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FEASIBILITY STUDY OF PHARMACOLOGICAL TREATMENT TO REDUCE MORBIDITY AND MORTALITY AFTER BRAIN INJURY

ANNUAL REPORT

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A single dose of d-amphetamine (AMPH) facilitates the rate of recovery of function in rats following contusion (400 g/cm) of the right sensorimotor cortex. In this report we present the behavioral, biochemical and metabolic results of several studies on recovery from contusion injury: 1) the effect of a systemic injection of morphine 24 hours post contusion injury on behavior, 2) the effect of contusion and AMPH treatment on brain norepinephrine (NE) and dopamine (DA) levels, measured with HPLC with electrochemical detection, 3) the effect of partial lesion of the locus coeruleus (LC) prior to contusion on behavior and brain NE levels, measured with HPLC with electrochemical detection, 4) the effect of intraventricular infusion of either NE or DA 24 hours following contusion injury on behavior, and 5) the effect of contusion and AMPH treatment on cerebral glucose metabolism, measured by the ^{14}C-2-deoxyglucose method. Animals were trained on a specific beam-walking task
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prior to each study. Behavioral recovery of function was measured by the animal's ability to perform on this task following the specific manipulations.

Morphine administration (10 mg/kg) 24 hours post-contusion facilitated recovery of function compared to saline control animals. HPLC analysis of catecholamines and their metabolites in rats 16 days after initial cortical injury showed a tendency toward decreased NE levels in the right cerebellum compared to sham controls. Injection of 6-hydroxydopamine into the LC bilaterally decreased NE levels bilaterally in frontal and cerebellar cortex as compared to sham controls. Recovery of the locomotor deficit was significantly faster for rats receiving LC lesions prior to contusion than for rats receiving only contusion. Intraventricular infusion of NE or DA produced no significant effect on recovery of function. Conclusions of the effect of contusion and AMPH treatment on brain glucose utilization cannot be drawn at this time because of the small number of animals on which data analysis has been completed.

The results of these studies support the hypothesis that at least one aspect of the mechanism of AMPH facilitation of recovery of function is via the catecholaminergic NE neurotransmitter system. (SDD)

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

Cerebral trauma results in severe and often permanent neurological disabilities[58] for which there is no accepted medical treatment that alleviates or even significantly diminishes the resulting deficits. However, recent work has suggested that short-term drug administration combined with appropriate experience produces an enduring enhancement of recovery of function.[24] Systemic administration of drugs that release norepinephrine (NE) such as amphetamine (AMPH) or alpha$_1$-adrenergic agonists, when combined with appropriate experience, facilitate recovery of function in several experimental models of cortical injury.[16, 21,22,24,33,50] The initial study used an ablation of sensorimotor cortex resulting in a contralateral hemiplegia on a beam-walking task. At 24 hours after surgery, a single administration of d-AMPH, when combined with relevant experience, results in accelerated recovery of beam-walking ability compared to saline controls. This acceleration of recovery appears at the first measurement one hour after drug administration and improvement persists beyond the period of drug action.[21] A recent pilot study using hemiparetic human stroke patients reported improved motor recovery when patients were given a single low dose of AMPH combined with physical therapy.[15,16] Importantly, this first clinical test of AMPH, which illustrates the usefulness of data derived from experiments using animals, was a double blind study with objective measures of motor function.

Several lines of evidence indicate that this d-AMPH induced facilitatiation of recovery of beam-walking after cortical injury occurs via modulation of hypofunction of energy generating metabolism in

morphologically intact regions[25,46,48,55,62] and/or alteration of catecholaminergic neurotransmitters affected by the lesion.[8,9,12,24,25] Additional pharmacological data specifically implicate the NE system in promoting recovery in the rat hemiplegia model.[10,11,24,55,59] As described in the previous Annual Report, in addition to results of other studies, some drugs (the $alpha_2$-antagonist, yohimbine)[61] were found to facilitate spontaneous recovery,[34,61] some ($alpha_1$-antagonist, prazosin) to retard recovery,[61] and others ($alpha_1$-adrenergic antagonists, prazosin and phenoxybenzamine, and the $alpha_2$-adrenergic agonist clonidine) to reinstate the symptoms of the injury after the animals had recovered.[23,34,61]

Several studies were described in detail in the first Annual Report which extended the above described finding to a model of focal cerebral trauma. Contusion of the right senorimotor cortex in rats produced a left hemiplegia which is measured on the beam-walking task, similar to the suction-ablation model. Also as in the ablation model, a single systemic administration of d-AMPH (2mg/kg) promoted recovery of beam-walking after rats received a 400 g/cm^2 injury. Animals injured with a 200 g/cm^2 impact showed rapid spontaneous recovery without AMPH administration whereas AMPH was ineffective in influencing the recovery of animals receiving 600 or 800 g/cm^2 injuries (even when given 3 drug administrations), as compared to saline control animals. Interestingly, d-AMPH administration did not affect recovery of the fine-motor task of grasping and retrieving a food pellet with affected digits. Evidently not all motor symptoms following cortical contusion are amenable to this treatment regimen.

The effect of seizures, which frequently follow contusion of the motor

cortex in humans,[18] on recovery in the rat hemiplegia model was then studied. We reported that two electroconvulsive shocks (ECS) facilitated recovery of animals receiving a 600 g/cm^2 injury, while 7 ECS's were ineffective. Because ECS may alter CA levels in brain, dependent on brain area and time of measurement relative to the seizure,[13,38,63] this effect of ECS on recovery may support the results of our pharmacological studies.

Our current emphasis has been to investigate the mechanisms by which d-AMPH facilitates recovery of locomotor function after cortical contusion. The effects of physiological and/or phamacological manipulations on the behavioral deficits of sensorimotor cortex contusion are being compared to biochemical changes of brain catecholamines and cerebral glucose utilization and oxidative metabolism. In this report we discuss behavioral, biochemical and metabolic results of several experiments using the 400 g/cm^2 sensorimotor cortex contusion model: 1) systemic injections of morphine following contusion, 2) the effect of contusion and AMPH treatment on brain catecholamine (NE and DA) levels, measured from several brain areas with HPLC electrochemical detection, 3) partial bilateral lesion of the locus coeruleus (LC) prior to contusion, with HPLC analysis of cortical and cerebellar NE levels, 4) intraventricular infusion of either NE or dopamine (DA) following contusion, and 5) examination of the effects of contusion and AMPH treatment on cerebral glucose metabolism was measured in several brain regions by the 2-deoxyglucose (2-DG) method developed by Sokoloff.[53]

GENERAL METHODS

Drugs. (-) Arternol bitartrate (NE), 3-acetylpyridine (DA), 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxy-phenylglycol (MHPG), d-amphetamine sulphate, pargyline, and 6-hydroxydopaimine (6OHDA) were obtained from Sigma Chemical. Merck Sharp and Dohme was the source of morphine sulfate. 2-^{14}C-Deoxy-d-glucose (300-350 mCi/mmole specific activity) was purchased from New England Nuclear.

Subjects. Rats weighing 250-350 g purchased from Harlan-Sprague-Dawley, were used in these experiments. Animals were housed individually in standard wire-mesh cages, maintained on a 12:12 hour light:dark cycle, and unless otherwise specified, given food and water ad libitum.

Apparatus. To test locomotor ability, a beam identical to that previously described in Feeney et. al.(1982)[21] was used. This apparatus consisted of a long (122 cm), narrow (2.5 cm) elevated (36 cm) wooden beam with a large, dark goal box similar in appearance to the animal's home cage attached to the end of the beam. A bright (60 watt) light source was positioned above the starting point and a speaker placed at the start position broadcasted a loud (approximately 62 dB) tape-recorded white noise. This noise was terminated when the animals entered the large (24.8 x 20.3 x 17.8 cm) goal box.

Surgery. All rats received either a contusion injury or served as sham operate controls. The procedure for producing a focal cortical contusion in the rat is described in detail elsewhere.[20] The apparatus consists of a stainless steel guide tube through which a weight was dropped to produce an impact force of 400 g/cm^2. The device was mounted on a stereotaxic carrier and the base of the device consisted of a stainless

steel circular footplate which the falling weight struck.

For surgery, all animals were given ketamine HCl (60 mg/kg, i.m.) as a preanesthetic, followed 10 min later by sodium pentobarbital (Nembutal, 21 mg/kg, i.p.). The scalp was shaved and cleansed with Ioprep and the animal placed in the stereotaxic apparatus. The scalp was opened and a craniotomy performed over the right hemisphere. The center of the footplate was positioned 1.5 mm posterior and 2.5 mm lateral to bregma. This represents the overlapping rat sensorimotor cortex, is relatively flat and accessible and when contused produces an observable deficit in beam-walking. The footplate was positioned so that it rested upon the surface of the intact dura. To prevent contused cortex from herniating through the cranial defect, craniotomies were made only slightly larger than the diameter of the footplate and after impact, the boneflap was replaced, sealed with bonewax and the scalp sutured closed. Sham-operates were treated identically except that no weight was dropped.

Beam-walk testing. One week (to allow recovery from transit and adaptation) after receipt and before surgery the animals were trained to traverse the beam. The first training day consisted of giving animals three non-rated trials using a successive approximation procedure. On trial 1 the rat was placed on the beam just outside the goal box; on trial 2 at the midway point on the beam; and on trial 3 at the start position. On the next day, and every other day thereafter, each rat received a single, rated trial on the beam from the start point. Locomotor performance and ability in traversing the beam was rated by two observers, one blind to all treatment conditions, using the 7-point rating scale described in Table 1. Criterion for the successful completion of beam-walk training was defined as achieving a presurgery score of "7" on

three successive trials.

Upon reaching criterion, rats received a 400 g/cm^2 contusion or craniotomy to the right sensorimotor cortex. At 24 +/- 1 hour postsurgery the animals were given a single trial on the beam to assess the severity of their disability. For most of the studies described in this report, unless otherwise noted, within five minutes following this test the animals received a single administration of drug [i.e., AMPH (2 mg/kg i.p.), morphine, or saline] and were returned to their home cage. Postdrug tests on the beam-walking task were given at 1, 2, 3, 6, and 24 hours and then every other day for 15 days unless otherwise specified. Beam-walking test sessions after the first day post surgery were conducted at the same time every other day.

Thionine histology. At the conclusion of the morphine, locus coeruleus lesion, and intraventricular infusion experiments each animal was given an overdose of Nembutal. When the animal no longer responded to the tail pinch, the thoracic cavity was entered and an incision made into the left ventricle and into the right atrium of the heart. The animal was perfused intracardially with 0.9% saline followed by a 10% formalin solution to fix the brain tissue. The brain was carefully extracted from the skull and placed in formalin.

Several days before the brains were to be cut, they were placed in a formalin and sucrose solution and placed in the refrigerator. When the brains had sunk to the bottom of the solution, they were prepared for cutting by mounting on a microtome chuck and freezing to -23^{o}C in a cryostat microtome (American Optical). Sections (40 um) of the brain were cut and every fifth slice mounted on a slide. After labeling the slides, they were stained with thionine.

Morphine

1) Dose response Study:

A total of 67 male Sprague-Dawley rats weighing 300-350 gm were used as subjects. Beam-walk training and contusion surgery were as described above. At 24 hours postoperatively, prior to any treatment, each animal was tested on the beam-walking task. In an effort to achieve an even distribution of animals according to severity of impairment within each treatment group, the severely impaired animals, i.e., those that scored a 2 on the beam-walk (see Table 1), were randomly placed in one of the four groups by the flip of a coin. Group placement for mildly impaired animals, i.e., those that had scored a 3, was also determined by the flip of a coin. Purely random placement might have resulted in one group containing a preponderance of severely impaired animals and another group containing more of the mildly impaired animals. It would then be more difficult to conclude that rates of recovery were the result of treatment and not due to a chance difference in degree of initial impairment between groups.

Animals in the control group (n = 9) received an i.p. injection of saline (SAL). Animals in the three treatment groups received i.p. injections of one of the following: 5 mg/kg MOR (n = 11), 10 mg/kg MOR (n = 9), or 20 mg/kg MOR (n = 8). The shams were randomly placed in one of the four groups: SAL (n = 3), 5 mg/kg MOR (n = 3), 10 mg/kg MOR (n = 4), and 20 mg/kg MOR (n = 4). The animals were scored on the beam-walk at twelve time-points post-injection: 1, 2, 3, 6, and 24 hours, and at 3, 5, 7, 9, 11, 13, and 15 days. Animals were weighed every other day both pre- and post-surgery and this was recorded to monitor general health. At the

end of each experiment (15 days post-surgery for experiments 1 and 2, 5-6 weeks post-surgery for experiment 3) brains were removed for histology. Drawings of each brain were made indicating the general location and dimensions of the contusions prior to sectioning and staining. (Results on p. 20).

2) Replication Experiment:

Twenty-two male Sprague-Dawley rats weighing 300-350 gm were used as subjects. Except for drug dose, all procedures, apparatus, and drugs used in this experiment were the same as those used in the first experiment. The four groups in this study were the severely impaired SAL, mildly impaired SAL, severely impaired MOR (10 mg/kg) and mildly impaired MOR (10 mg/kg). On the beam-walk test at 24 hours post-injury, a beam-walk score of 2 was used to indicate severe impairment and a score of 3 indicated mild impairment.

Twenty-four hours following surgery, animals that scored a 2 on the pre-drug beam-walk were randomly placed into either the 2-SAL group (n = 6), or the 2-MOR group (n = 6). Animals who scored a 3 were randomly placed into either the 3-SAL group (n = 5) or 3-MOR group (n = 5). The 2-SAL and 3-SAL groups received i.p. injections of SAL; the 2-MOR and 3-MOR groups received i.p. injections of 10 mg/kg MOR. Each animal was tested on the beam-walk at 1, 2, 3, 6, and 24 hours after injection and then every other day until day fifteen. (Results p. 21).

3) Reinstatement experiment:

Six of the recovered control animals from experiment 1 were held for two weeks following the 15th day beam-walk and tested again to be sure they had maintained their level of function at a score of 7. Three of these animals had scored a 2 on the pre-drug beam-walk and three had

scored a 3. All six of these animals were given an i.p. injection of 10 mg/kg MOR and then scored on the beam-walk at the same time points as in experiment 1.

The 10 mg/kg dose of MOR was selected to test for reinstatement because on post-hoc analysis of the data from the first experiment, it was found to be the dose that influenced locomotor recovery. Also, none of the beam-walk scores for shams that had received this dose dropped below a 7 at any time after injection. Since all of the shams that had received 20 mg/kg dropped to a 2 on the beam-walk at one hour post-injection, it would not be possible to differentiate between a simple drug effect and reinstatement of deficit with this dose. (Results p. 22).

HPLC

Rats were killed by decapitation 16 days after contusion injury and their brains were rapidly removed and dissected over ice into right and left frontal cortex (CX), cerebellar cortex (CB), locus coeruleus (ML), and ventral tegmental (VT; including substantia nigra) areas. Tissue was immediately frozen at -80^{o}C and saved for assay. Just before assay brains were weighed and sonicated for 9 sec (Fisher Model 300 sonic dismembrator, setting 45) in 20 volumes of pH 4.0 30% acetonitrile buffer with isoproterenol (.3ng/ul) added as internal standard. The homogenate was centrifuged at 11,000 xG for 10 min at 4^{o}C. The clear supernatant was then injected directly into the HPLC.

Norepinephrine (NE), dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were quantified by reverse-phase high performance liquid chromatography with electrochemical detection (a BAS LC-48 amperometric detector equipped with a glassy carbon working electrode and a $Ag/AgCl_2$

reference electrode). The column was an Alltech Adsorbosphere C18 reverse-phase column, protected by an Alltech guard column. NE, DA, and DOPAC were eluted by a 9% acetonitrile-water mobile phase (v/v), pH 4.35, containing 1.0 mM EDTA, 0.1M $NaH_2PO_4.H_2O$, and 0.7 mM SOS. The flow rate was 1.0 ml/min at room temperature. Single electrode detection was performed at an oxidation potential of +0.8 V. Compounds were plotted and quantified automatically by a Hewlett-Packard 3390A integrator. This system produced very good separation of all compounds from each other and from the internal standard. Standard curves were run for each compound. A minimum concentration of 1.5 pg/ul of injected standard was detectable.

The NE metabolite 3-methoxy-4-hydroxy phenylglycol (MHPG) was not resolveable on the same run we used to detect the amines. We therefore extracted (with ethyl acetate) 25 ul of the same sample that was prepared for the amines. To each 25 ul of tissue homogenate supernatant, 100 ul of ethyl acetate was added, the sample vortexed, and the supernatant pipetted off and saved. The 25 ul sample was further extracted two more times using the same procedure. This left approximately 300 ul of ethyl acetate supernatant containing MHPG, which was then evaporated under a nitrogen stream and following evaporation was immediately resuspended in 20 ul of homogenization buffer and vortexed thoroughly. In standards we were able to recover 90-95% of free MHPG with better recovery at the lower concentrations.

A total of 26 rats were prepared for the 16 day time-point study. Either AMPH or saline was administered 24 hr. following contusion or sham surgery. Animals were decapitated 16 days after surgery (15 days after drug injection). The brain samples were prepared as described above and levels of NE, DA, DOPAC, and MHPG were measured. (Results on p. 22).

Locus Coeruleus Study

Fifty male Sprague-Dawley rats weighing between 250-300 g at the time the LC was bilaterally lesioned and between 300-350 g at the time the right sensorimotor cortex was contused were used. Once beam-walk training was completed animals received either bilateral lesions of the LC or a sham operation. All animals were food deprived 16 hours prior to this stereotaxic surgery and anesthesized with a combination of ketamine (i.m.; 60 mg/kg) and atropine (i.m.; .08cc) followed 10 minutes later with Nembutal (i.p.; 30 mg/kg). Animals were also given the MAO inhibitor pargyline (i.p.; 50 mg/kg) 5 min after the ketamine/atropine injection, to enhance the effectiveness of the 6OHDA LC lesions. The LC lesions were made by the local infusion of 2.5 ul of 6OHDA solution at stereotaxic coordinates given below. The vehicle for 6OHDA consisited of 0.9% NaCl solution containing 1 mg/ml ascorbic acid as an antioxidant. Thus, 10 ug of 6OHDA base was freshly (i.e., kept in freezer in base form) dissolved in 5 ul of the saline/ascorbic acid (i.e. 2 ug 6OHDA/ul saline/ascorbic acid) for each animal immediately prior to infusion. Half of the 6OHDA solution (2.5 ul) was slowly infused (.50 ul/30 sec) into each side of the LC through a beveled needle (open side facing forward toward bregma) attached to a Hamilton syringe. The needle remained in the LC for 2 min after completing the infusion to allow the 6OHDA to diffuse into the region of the LC. Two sets of coordinates were employed in this study. The first set of coordinates were those used by LaManna et. al. (1981);[31] 1.1 mm posterior, 1.3 mm lateral, and 7.5 mm ventral (from skull) to lambda. The second set was a modification of the same LaManna coordinates; 1.2 mm posterior, 1.3 mm lateral, 7.0 mm ventral (from skull)

to lambda. The second set of coordinates was used because histology on brains lesioned using the first set of coordinates suggested the lesion was too far anterior to destroy most of the LC. Therefore, the latter coordinates were tested in an attempt to produce a more extensive LC lesion. Sham operates were treated the same as lesioned rats but no penetration of dura was made.

Two weeks were allowed for a degeneration of LC fibers after 6OHDA cell body lesions (see Fig. 1 for a diagram of the paradigm used in this study). During this time all animals received 4 training trials on the beam in order to evaluate the effect of the bilateral or sham LC lesions on the beam-walk task. One trial was given 24 hours after sham or LC lesion and then one trial was given every three days. On day 15, animals received a contusion to the right sensorimotor cortex or a sham operation. For this surgery, animals were not food deprived and received a slightly higher dose of Nembutal (i.p.; 30-45 mg/kg).

In order to maintain consistency with our previous pharmacological experimental protocol, animals were tested on the beam walking task 24 hours after receiving a contusion or sham operation and then 1, 2, 3, and 6 hours after the 24 hour time point (even though no drug was administered at 24 hr. post contusion) in this study. This was considered important because our data examining drug effects on this model indicate the essential role of experience on the beam-walking task.[21] Testing occurred again 48 hours after contusion and then every other day for 16 days.

All animals were killed by decapitation 24 hours after the last beam-walk trial, 16 days after contusion or sham surgery (Fig. 1). The brain was quickly removed and cut into rostral and caudal sections. The

brain stem was placed in formalin for histological evaluation of LC lesions. Tissue samples were quickly taken from both hemispheres of the frontal and cerebellar cortex of the remaining section and frozen for HPLC analysis of NE levels. (Results on p.24).

NE and DA Intraventricular Infusion.

One hundred forty-six rats weighing 300-325 g were used in this study. Following the standard beam-walk training, the evaluation of the effects of the intraventricular infusion of NE or DA on recovery from cortical contusion was conducted in two stages. On the day of contusion surgery rats were anesthetized with sodium pentobarbitol (Nembutal; 21 mg/kg, ip.) preceded by a mixture of ketamine hydrochloride (Ketalar; 60 mg/kg,im.) and atropine (.08 cc, im.). During this surgery session the rats received a contusion injury to the right sensorimotor cortex. A hole was also drilled over the left lateral ventricle (1 mm posterior and 1mm lateral to bregma), and sealed with bonewax for the intraventricular injection on the following day.

The intraventricular infusion surgery was conducted twenty-three hours later. Ether anesthesia (Ethyl Ether, Reagent A.C.S.) was used for this surgery making it possible for the rat to be tested on the beam-walk task at the one hour time point. The sutures were cut, the scalp reopened, and the bonewax was removed from the intraventricular injection hole. NE or DA was then infused into the ventricle, using Hamilton (Reno, Nv.) microsyringes. Doses of NE and DA were 50, 100, 150, 200, and 400 micrograms/10 microliters.

The syringe was centered over the hole and lowered 5 mm from the skull into the ventricle. Ten microliters were slowly injected (by hand) over a

two minute period, then one minute was allowed for diffusion and the syringe slowly removed over a one minute interval. The hole was resealed with bonewax and the scalp sutured closed. The standard routine for behavioral testing was begun one hour after suturing the scalp. (Results p. 26).

2-Deoxyglucose Utilization.

A total of 65 rats were prepared for this study, weighing 300-350 g. At 2, 6, or 16 days post contusion surgery rats were catheterized in the femoral vein and artery for the injection of 2-DG and drawing of arterial blood samples. Halothane anesthesia (Halocarbon Laboratories Inc., Hackensack, N.J.) was induced within a bell jar using a Fluotec Mark 2 vaporizer (Cyprane Ltd., Kerghley, England) set at 4% with an O_2 flow rate of 4 liters/min for 3-5 min. The rat was shaved in the femoral area and scrubbed with betadine and then placed in a nose cone with rebreathing bag and gas filter system (Bickford Inc., Wales Center, New York). O_2 gas flow was lowered to 1.5 liters/min and halothane was lowered to 1.5 to 2.0% for the maintenance of anesthesia during surgery.

The femoral vein and artery were surgically exposed for catheterization. Polyethylene PE-50 tubing I.D. 0.58 mm, O.D. 0.965 mm approximately 30 cm long and connected to a 1 ml syringe filled with heperinized normal saline, 1 ml of 1,000 USP units/ml heparin in 100 ml normal saline was used for the femoral artery catheter. The catheter was beveled at 45^{o} and inserted into the lumen of the artery 1.25 to 2.0 cm with a 0.2 mm tipped vessel dilator (Fine Science Tools Inc., Belmont, Ca.). Silastic tubing 0.512 mm I.D., 0.937 mm, 30 cm long (Dow Corning Corp., Midland, Mi.), beveled at 45^{o}, was inserted 4.0 to 5.0 cm into

the femoral vein. Both catheters were held in place with three 5-0 silk ties around each vessel. The area of the incision was infused with 0.3 ml of lidocane and the incision closed with 4-0 prolene, with both catheters exiting at the posterior end of the incision. Aseptic techniques were used throughout this procedure.

When the rat was removed from anesthesia it was placed in a restraint device 28 cm long with a beam 3.5 cm wide by 1.5 cm thick raised 10 cm off the floor. At both ends of the restraint device is a circle of seven 0.75 cm holes. The circle is the diameter of the rat's body and allows dowels to enclose and restrain the rat. The rat was taped to the beam with 1" adhesive tape securely enough to limit movement but allowed unrestricted breathing. All four legs are taped to the floor of the restraint device to limit pedal movements.

The rat was allowed to recover from the effects of anesthesia for 2 hours post surgery before the injection of radioactive substrate. 2-DG was evaporated under a fume hood and reconstituted in 0.30 ml normal saline. After an initial (time 0) arterial blood sample was drawn the 2-DG was injected in the venous line as a bolus followed by 0.7 ml of normal saline to flush the line. Arterial blood samples (200-400 ul volume) were taken at 0, 20, 40, 60 sec., 1.5, 3, 5, 7.5, 15, 25, 35, and 45 min. Plasma from these samples was used to measure blood levels of 2-DG and glucose. Both parameters are needed to calculate local glucose utilization. Levels of 2-DG are measured by scintillation counting of all samples and blood glucose was measured in the 0, 3, 7.5, 25 and 45 min. samples with a glucose assay kit (Sigma), based on determination with glucose oxidase.

To ensure normal physiological condition of each rat after these

procedures, measurements of blood pH, pCO_2, pO_2, and plasma glucose and corticosterone levels were taken from the 45 min. samples. Animals with abnormally low or abnormally high blood pH and/or blood gas levels were discarded from the study.

The rat was then decapitated and its brain quickly (1-1.5 min) removed and placed in isopentane cooled to -30°C in an acetone bath containing dry ice. The frozen brain was then embedded in O.C.T. compound (Miles Scientific, Naperville, Il.) and placed in a subzero freezer at -80°C until cutting on a cryostat.

Brains were cut into coronal sections 20 microns thick and every tenth section was sampled for autoradiographic analysis. Each section was thaw-mounted onto clean microscope glass slides, let dry for at least 24 hours, and apposed to Kodak NMC x-ray film for three weeks. Kodak NMC film is optimal for giving the best signal-to-noise ratio with an exposure time of three weeks. (Results p. 27).

RESULTS

Morphine.

1) Dose response experiment.

Initial statistical analysis revealed no significant difference in the rate of recovery among the four groups over the 12 time points ($F = .98$; $d.f. = 3, 32$; $p = .413$), (see Fig. 2). A post-hoc analysis of the data was done comparing the severely impaired animals (pre-drug score 2) with the mildly impaired animals (pre-drug score of 3). This analysis, which evaluated only the 6 hr to 3 day time points (the time period after drug intoxication and before spontaneous recovery had occurred), did reveal a statistically significant facilitation of recovery in the 10 mg/kg MOR

animals that were mildly impaired compared with the SAL animals that were mildly impaired ($F = 10.79$; d.f. = 1, 8; $p = .011$).

The severely impaired animals in the 10 mg/kg group recovered at the same rate (from 6 hours to 3 days) as the severely impaired animals in the SAL group ($F = .00$; d.f. = 1, 6; $p = 1.000$). Figures 3 and 4 illustrate the difference in recovery rates between severely and mildly impaired groups.

Comparison of the beam-walk scores for the severely vs. the mildly impaired animals in the 10 mg/kg MOR group yielded the following values: $F = 57.68$; d.f. = 1, 8; $p < .001$ (6 hours to 3 days) and $F = 15.69$; d.f. = 1, 7; $p < .005$ (1 hour to 15 days). The 10 mg/kg MOR group was the only group which demonstrated a difference in recovery rate between the mildly and severely impaired animals. For the mildly vs. the severely impaired animals in the SAL group (6 hours to 3 days) $F = 1.94$; d.f. = 1, 7; $p < .206$; and $F = 1.69$; d.f. = 1, 7; $p < .235$. (1 hour to 15 days).

All of the 14 sham animals scored a 7 on the pre-drug beam-walk. All of the animals that received SAL (n = 3), 5 mg/kg MOR (n = 3), and 10 mg/kg MOR (n = 4) continued to score 7 on the post-drug tests. However, all of the animals that were given 20 mg/kg MOR (n = 4) were unable to traverse the beam at one hour post-injection, scoring a 2. Three of these four animals scored a 7 at 2 hr post-injection, whereas one of them scored a 6 at 2 hr but scored a 7 at 3 hr. Review of the experimenter's notes of subjective observations taken during the beam-walk indicate that the animals that had received 20 mg/kg MOR were described as appearing sedated 2 to 3 hr post-injection even after they were able to score a 7 on the beam-walk. No subjective signs of intoxication were noted at 6 hr post-injection.

2) Replication study.

Because a post-hoc analysis of the data from the 10 mg/kg MOR group revealed a difference in recovery rates between mildly and severely impaired animals, a second study was carried out to determine if these results could be replicated.

As in Study 1, a significant facilitation of recovery ($F = 5.70$; $d.f. = 1, 8$; $p < .044$) was seen between 6 hours and 3 days in the mildly impaired animals that received 10 mg/kg MOR when compared with the mildly impaired animals that received SAL. Again, there was no difference in recovery rate between the severely impaired animals that received SAL and those that received MOR ($F = .65$; $d.f. = 1, 10$; $p = .440$). The 3-MOR group recovered significantly faster than the 2-MOR group from 6 hours to 3 days ($F = 57.68$; $d.f. = 1, 9$; $p < .001$) as well as from 1 hour to 15 days ($F = 45.36$; $d.f. = 1, 9$; $p < .001$). The 3-SAL and 2-SAL groups recovered at essentially the same rates from 6 hours to 3 days ($F = 1.68$; $d.f. = 1, 9$; $p < .228$) and from 1 hour to 15 days ($F = 3.15$; $d.f. = 1, 9$; $p < .110$).

3) Reinstatement study.

All six animals continued to score 7 on the beam-walk both during intoxication and after the drug effect had worn off (data not shown). The study was discontinued since no deficits were seen in two animals after ten trials, two animals after seven trials and two animals after five trials.

HPLC (16 day time point)

NE and MHPG

The samples analyzed were taken 16 days post injury (15 days post

drug or saline control). As shown in Figure 5 and Tables 2-4 we found similar right-left levels of NE in the locus coeruleus (LC), substantia nigra (VT) and cerebellar cortex (CB) with highest levels in the locus coeruleus and lowest in the cerebellar samples. None of these areas in either hemisphere showed significant effects of any treatment on NE levels. Fig. 5 suggests that injury or AMPH alone or injury and AMPH increased NE levels in the left LC compared to the control (sham/saline) but this effect was not significant.

Although NE levels did not clearly indicate any differences among the treatment groups, the concentration of the major NE metabolite, MHPG, in the cerebellum did reflect some enduring effects (Table 4). MHPG concentrations in the cerebellum contralateral to the injury (left) were quite stable across all 3 treatment groups and the sham control group, indicating no effect of either injury or AMPH on levels of this metabolite. However, MHPG levels in the cerebellar cortex ipsilateral to the injury were significantly higher compared to the sham control group. However, this control group had very low MHPG levels compared to the opposite cerebellum and may not represent a normal sample. No other statistically significant differences were found.

As shown in Table 4 the ratios of the means of NE/MHPG show large differences between hemispheres in the control groups making it difficult to compare the injured to the non-injured hemisphere. It appears that the ratios are quite consistant in the cerebellar cortex of the uninjured side (2.1-2.5) but not in the cerebellum ipsilateral to the injury. There is some suggestion (not significant) that turnover of NE is increased with injury (1.6) which may be somewhat reversed with AMPH (3.5) toward the non-injured AMPH state (4.2).

DA and DOPAC

As seen from Table 5, DA and DOPAC levels were measurable in the right and left substantia nigra, but not in cerebellar cortex nor LC. There were two animals in the injury/AMPH group that did show small but measurable DA levels, and one of these also showed measurable DOPAC.

DA levels were similar in the right and left substantia nigra with no significant differences among any of the treatment groups; indicating no enduring effect of either AMPH or injury on DA levels. Similarly there were no differences in DOPAC levels between treatment groups. DOPAC concentrations were below measurable levels in both right and left LC and cerebellum.

As can be seen in Table 5 the DA/DOPAC ratios were quite consistant between hemispheres for the control groups (sham/saline). Administration of AMPH to the controls led to a decrease in this ratio which was more pronounced on the right (injured) side than in the uninjured side, which suggests increased DA turnover. However, the AMPH effect in injured rats was not the same as in uninjured rats as AMPH increased the ratio on the right and decreased the ratio on the uninjured side, suggesting decreased DA turnover when AMPH is given to injured rats. Injury alone did not appear to alter the ratio of DA to DOPAC.

Locus coeruleus lesion.

Histology: Of the 31 animals that received a bilateral lesion of the LC with either set of coordinates, 8 brains displayed visible damage to the anterior and midportion of the LC.

Behavior: Behaviorally, there was no difference between animals for the different coordinates used to bilaterally lesion the LC. Therefore,

these data were combined and these animals were labeled Group Lesion & Contusion (n = 31). Those animals that received a sham bilateral LC lesion followed by a contusion were labeled Group Contusion Only (n = 7). Animals that received a bilateral LC lesion followed by a sham contusion were labeled Group Lesion Only (n = 7) and those animals that received a sham bilateral LC lesion followed by a sham contusion were labeled Group Sham-Sham (n = 5). The behavioral data from these four groups are illustrated in the Fig. 8.

A repeated measures analysis of variance performed on these data revealed a significant effect of group, $F(3,46) = 22.10$; and time, $F(11,506) = 108.86$; as well as a significant interaction of these two variables, $F(33,506) = 13.43$ ($p < .00$ in all cases). Subsequent simple effect analyses revealed that it was at the 1 [$F(3,46) = 111.94$)], 2 [$F(3,46) = 26.27$], 3 [$F(3,46) = 20.43$], and 6 [$F(3,46) = 16.96$] hour and the 2 [$F(3,46) = 11.76$] and 4 [$F(3,46) = 3.90$] day time points that the groups significantly differed (all $p < .05$).

In order to further analyze differences among the individual group means at these six time points, the Newman-Keuls statistic was employed. According to this test, Groups Lesion Only and Sham-Sham did not differ at any of these time points. Animals in these groups exhibited perfect performance (i.e., all 7s) on the beam. These two groups displayed significantly better performance on the beam-walking task than did either of the remaining two groups at all time points except day 4. On day 4, these two groups displayed significantly better performance on the beamwalking task than did Group Contusion Only. Interestingly, Group Lesion & Contusion performed significantly better on the beam than did Group Contusion Only only at the 3 and 6 hour time points. No other

comparisons reached significance.

Biochemical Results.

Frontal Cortex: As illustrated in Figures 9 and 10 there was no significant interaction effect nor was there a difference between hemispheres. However, it was found that there was an overall groups effect ($F_{4,71} = 4.14$, $p < .0049$). Newman-Keuls tests revealed that the Sham-Sham group differed from both the Lesion + Contusion and Lesion groups indicating that the LC lesion and the LC lesion with contusion each significantly decreased NA in frontal cortex. However, closer inspection of the data indicated that the contusion may have depleted NE in the right frontal cortex, an effect that may have been obscured by a smaller decrease in the left frontal cortex. It appeared this was the case as a t-test showed a significant depression of NE in the right frontal cortex as compared to Sham controls ($t_2 = 2.88$, $p < .05$). Compared to the Sham controls NE was depleted 57% in the Lesion + Contusion group, 54% in the Contusion group, and 53% in the Lesion group in the right hemisphere. Left hemisphere depletions were lower as shown in Figures 9 and 10. In this hemisphere there were 38%, 27%, and 47% depletions for the Lesion + Contusion, Contusion and Lesion groups respectively compared to the Sham controls.

Cerebellar Cortex: Analysis of variance showed no statistically significant interaction between variables, and no difference between hemispheres in NE concentrations indicating no difference between injured and non-injured sides. However, NE was significantly decreased in some groups ($F_{4,69} = 28.9$, $p < 0.0001$). As can be seen in Figures 11 and 12 and confirmed by Newman-Keuls tests both Lesion + Contusion and Lesion groups differed from the Normal, Contusion, and Sham control groups which

did not differ from one another. Unlike the frontal cortex the Contusion group did not differ from the Sham controls. In right cerebellar cortex there were decreases of 80%, 30%, and 69% for Lesion + Contusion, Contusion and Lesion groups respectively. The percent depletion compared to Sham controls in left cerebellar cortex were 82%, 25%, and 82% for the three groups.

Intraventricular infusion.

Behavioral data for intraventricular infusion of NE and DA are shown in Fig. 13 and Fig. 14, respectively. A repeated measures analysis of variance was performed on the beam-walk data for the animals that received NE. It revealed a significant effect of time, $F(11,594) = 238.19$ $p < .0005$; with the group effect being nonsignificant $F(5,54) = .75$ $p < .589$. However, there was a significant interaction between these two variables $F(55,594) = 1.90$ $p < .0005$. The DA data analysis showed a significant effect of time, $F(11,374) = 103.93$ $p < .0005$; and a nonsignificant group effect $F(5,34) = 1.89$ $p < .122$; and significant interaction between these two variables $F(55,374) = 1.59$ $p < .007$, also. A simple effects analysis revealed that it was the 1 hr $F(5,59) = 6.44$ $p = .001$ and Day 5 $F(5,59) = 2.75$ $p = .028$ time points that were significantly different for the NE data set. The 3 hr $F(5,39) = 2.63$ $p = .041$ time point was significantly different for the DA data set. A Newman-Keuls analysis was performed to analyze the individual differences among the group means. It was found that, for the NE group, saline significantly differed from the 100, 150, 200, and 400 microgram/10 microliter groups and the 50 microgram/10 microliter group significantly differed from the 150, 200, and 400 microgram/10 microliter groups at the

one hour time point. Also, there was a significant difference between the 100 and 200 microgram/10 microliter groups at day seven. The Newman-Keuls analyses of the DA data revealed no significant differences between groups; even though the simple effects analysis showed significance at the 3 hr time point.

2-Deoxyglucose Utilization.

The ranges of the five variables we have thus far are as follows: pH, 7.22 - 7.51; pO_2, 96.9 - 134.0; pCO_2, 28.7 - 42.3. Plasma glucose levels (mg/dl) at time point zero min., 7.9 - 45.0; at three min., 12.5 - 31.0; at 7.5 min., 10.5 - 40.0; at 25 min., 10.5 - 41.5; and at 45 min., 14.8 - 36.0. These results suggest that our animals were within the normal physiological range. However, the levels of corticosterone suggest that all our animals were under stress throughout the procedure. Before infusion of 2-DG and withdrawing of blood samples, corticosterone levels ranged 278 - 486 ng/ml and immediately after all the samples were drawn, the corticosterone levels ranged 184 - 746 ng/ml. This unexpected result has important implications for the widely used 2-DG method.

Because autoradiography has been quantitated for only two rats per group thus far, it is impossible to perform any statistical tests and draw any conclusions. However, preliminary visual inspection of the data (Table 6) reveals that in the substantia nigra (SN) ipsilateral to the injury, compared to the contralateral SN, there is a large reduction in local cerebral glucose utilization in saline-treated rats compared to their sham counterparts. This enduring effect of injury was not seen in the two AMPH-treated contused group. A similar pattern was seen in the dorsal tegmental nucleus. Finally, there is a substantial effect of

injury between heispheres in the posterior neocortex. The cortex ipsilateral to the contusion showed a marked reduction of glucose utilization compared to the contalateral hemisphere.

DISCUSSION

The effects of morphine on recovery of function were unexpected. Based on other studies of infarct models and stroke cases, we expected that morphine would retard recovery of beam-walking after contusion.[6,32] However, the animals that were moderately disabled (had a predrug score of 3, see Table 1) by the contusion injury and received a 10 mg/kg dose demonstrated improved rates of recovery compared to the saline controls during the 6 hour through 3 day time period. This effect was puzzling since 5 and 20 mg/kg had no effect and the 10 mg/kg dose did not imporve recovery of function for more severely disabled animals (a predrug score of 2). However, this post hoc observation was replicated. The lack of effect prior to the 6 hr. time point was likely due to morphine's initial sedative effect. A dose of 10 mg/kg did not increase the rate of recovery of the severly injured animals, indicating that severity of injury is an important factor in determining the effects of morphine on recovery of function. This may account for some of the conflicting reports on the effects of morphine on recovery of function in the literature. Also, there was no significant difference between recovery of beam-walking of the moderately versus the severely disabled animals in the saline control groups. Apparently this results from the extent of variability in the moderatly disabled animals.

In another aspect of the morphine study, administration of 10 mg/kg 2 weeks following spontaneous recovery (and 4 weeks post-contusion

injury) did not reinstate symptoms. No deficit was displayed on the beam walking task in spontaneously recoverd rats. However, an enhanced/accentuated deficit was noticed in approximately 50% of contused rats at the 1 hour time point following morphine administration (10 mg/kg), when given 24 hours after contusion, and this was significantly different from saline controls. No sham operated control animal given this dose of morphine demonstrated a drop in performance at the one hour time-point. With the present data it is not possible to firmly establish if the depressed functional ability at 1 hour, occurring in some morphine and saline treated animals, is due to drug intoxication or reinstatement/enhancement of the functional deficit. The absence of depressed beam-walking performance in morphine-treated sham animals argues against an explanation of simply sedation of contused animals. Therefore, it is possible that morphine could reinstate or exacerbate symptoms if given very soon after contusion in the rat. Reinstatement of functional deficit by morphine administration may be dependent upon the time of administration relative to the time of injury. Baskin et. al.[5] report morphine reinstated neurologic symptoms in one patient, after spontaneous recovery from an ischemic stroke. Other animal models of ischemic stroke report morphine reinstates functional deficits,[6,32] if the drug was given within 7 hours of the initial ischemic injury.

These effects of morphine could be related to release of cerebral NE. There are conflicting reports regarding the action of morphine on the LC. Morphine is reported to inhibit firing of the LC both in an in vitro study using rat brain slices[3] and in an in vivo study in anesthetized cats.[54] The inhibition of firing is due to the agonist action of morphine on opiate receptors. Conversely, Trulson and Arasteh[57] report

morphine to increase activity of LC neurons, based on in vitro studies with mouse brain slices. The most recent data using unanesthetized and freely moving animals[49] also found morphine increased LC unit activity. These authors speculate that the conflicting results are due to morphine's interaction with anesthesia. Our finding that morphine enhances recovery of function could be interpreted as an action on the LC neurons. If morphine induces release of cerebral NE by increased LC firing, this would be compatible with the interpretation that AMPH affects recovery of function by enhanced release of NE. Why this only occurs for mildly disabled animals and the suggestion that morphine, in some situations, can reinstate symptoms remains to be elucidated.

The lesions of the LC that decreased frontal NE concentration by 50% had no effect on the beam-walking task. The unexpected finding was that rats given a contusion in the right cortex showed as great a decrease of NE in frontal cortex as did rats given a lesion followed by contusion, or a lesion alone. The fact that lesioned-only rats showed no deficit on the beam-walk task, but that rats given a contusion that decreased frontal NE to the same level as lesioned rats, but did show a deficit on beam-walking indicates that damage to forebrain NE terminals per se is not responsible for beam-walk deficits produced by contusion.

The decrease in frontal NE is quite long lasting as we measured NE 30 days post lesion and 16 days after sensorimotor cortex contusion. This long-lasting decrease in NE is partially in agreement with Robinson et. al. (1980)[51] who found 49% decreases in ipsilateral frontal cortex eight days after middle cerebral ligation. At 16 days post-ligation these authors report a small decrease (about 60% of control levels) in NE concentration which is a somewhat below our reports of about 50%.

However, these differences could easily be explained by the differences between inducing brain injury by ischemia vs. direct cortical injury. The extent and uniformity of the decrease in NA concentrations in the cortex ipsilateral to injury was greater than in the contralateral cortex. This may have been partially due to the fact that the tissue samples were taken from a site too near the injury. With a contusion injury there is the possibility of the cortex becoming undercut and thus distorting measurements. We are planning to extend these findings by testing other parts of cortex removed from any direct effects of the injury.

The effects of contusion on cerebellar NE were not as marked as those in cortex, but we still found a tendency for lowered NE levels in the group given only contusion as compared to sham controls in the cerebellum contralateral to injury. These findings indicate that contusion is capable of leading to NE decreases in sites remote from, but neurochemically connected to the injury. In coroboration with the findings in frontal cortex it appears to be the case that lowered NE levels in cerebellum are not the reason for deficits on the beam-walk task. We have to a certain degree replicated these biochemical findings in cerebellum where we find a 65% depletion of cerebellar NE in the cerebellum ipsilateral to the injury with no depletion in the cerebellum contralateral to injury.

Animals given a partial bilateral LC lesion 2 weeks prior to 400 g/cm^2 contusion demonstrated significantly enhanced recovery compared to rats that received only a contusion. This was significant for only the 3-6 hour time points after contusion. These data are likely a result of processes such as denervation supersensitivity or sprouting[26] of the LC during the 2 week time period between the LC lesion and the

cortical contusion. Such events could increase the sensitivity or amount of NE in other brain areas.

Either NE or DA were intraventricularly administered 24 hours following cortical contusion to clarify the roles of these catecholamines in recovery of function. Unlike AMPH, neither produced a statistically significant enduring improvement in recovery of beam walking compared to the saline controls. Infusion of NE was expected to facilitate recovery because Boyeson and Feeney[10] reported that intraventricular administration of NE improved recovery of function after sensorimotor cortex ablation. The lack of effect of NE or DA in our experiments could be due to unknown differences between these injury models. Perhaps forces from the contusion alter NE receptors, affecting their response to the catecholamines. Therefore, drugs that have longer lasting effects may be more effective shortly after contusion than the actual transmitter itself. Two other aspects of our results require further study before rejecting a hypothesis that the infusion of the catecholamines (particularly NE) are effective. First, inspection of Fig. 14 suggests a facilitated recovery compared to the saline controls for the 150 ug dose of DA. This experiment should be repeated to see if the trend is significant with a larger sample size. Second, a different behavioral effect was apparent between the use of old versus new NE (data not shown). A lower dose of new NE (100 ug/10ul; n = 2) suggested a facilitated recovery. The profile of 150 ug/10ul (n = 2) of old NE is virtually identical to the 100 ug/10ul dose of the new, which could be interpreted as indicating that the old NE has a decreased potency compared to the new. The 200 ug/10ul (n = 2) of old NE appears promising 2 hrs after infusion, but then lacked an enduring affect on recovery.

Unfortunately, the results are not significant because of the small number of animals in each of the above groups. A wide range of doses had to be studied in order to determine the limits for a subsequent dose response study. However, a differential effect appears to be present which is dependent on the age of the drug batch and how soon after purchase it was used. Because NE will oxidize over time and such small amounts are used in intraventricular infusion, this could alter its behavioral effects. The NE batches were returned to Sigma for chemical analysis, who reported no change since the compounds were initially shipped. Their analysis was based on infrared spectroscopy (IR) and thin layer chromatography.

Similar to the behavioral differences from the different batches of NE, behavioral differences were also obtained from different batches of AMPH. In the first year of work we had initially seen a significant difference in the recovery of AMPH treated versus saline control animals in behavioral recovery after 400 g/cm^2 contusions. However, in later studies (data not shown) the behavioral effect following administration of AMPH diminished. We have since repeated the behavioral study with a newly acquired lot of AMPH (Sigma), and confirmed the results in the well established suction-ablation model. One advantage of the suction-ablation model is that the injuries are more consistant from animal to animal, whereas the extent of the injury and resulting disability in the contusion model are more variable. The newer batch of AMPH (manufactured 4/87, received 12/87) significantly facilitated recovery of animals on the beam walking task during the period of intoxication, as compared to animals injected with the older batch of AMPH (manufactured 9/84, purchased 1/86). Smith-Klein and French laboratories chemically analyzed the both lots with IR, nuclear magnetic resonance (NMR, ^{13}C and H^+) and

ultraviolet (UV) analysis. Only the UV analysis showed a contamination present in the older batch, which has not yet been identified nor quantified, but is thought possibly to be a polyphenol compound. The presence of this contaminate, in addition to possibly producing its own biological activity, would also decrease the actual amount of AMPH administered to the animals, depending on the contaminant's concentration relative to the entire sample.

The results of the 2-DG study suggest that the animals have normal blood glucose, blood gases and pH values but are stressed by some aspect of the method. Stress was determined by measuring corticosterone levels. To our knowledge no one has studied the stress induced by this widely used technique. The importance of this observation for interpreting the autoradiographs is critical for our hypothesis because stress, induced by only 15 min. of restraint, has been shown to increase *LC* neuronal activity in cats.[1] Although their model of restraint was more extreme than ours, it is possible that the Sokoloff[53] experimental procedure which requires restraint also induces alterations in LC firing. This could affect apparent glucose utilization on 2-DG autoradiograms. However, bearing in mind that all 2-DG data may come from stressed animals, interesting results may arise from comparing the metabolism of saline versus AMPH treated sham and contused animals. All animals undergo the same surgical procedure and restraint prior to injection of ^{14}C-2-DG. Even if the restraint itself is producing stress but only mildly altering cerebral metabolism, we may be able to delineate drug effects on glucose metabolism. We are in the process of determining at what point during the 2-DG procedure (anesthesia, femoral catheterization, restraint) the corticosterone levels increase. It is important to consider the

limitations of 2-DG studies in models of brain injury. These limitations result from the assumption that rates of glucose and 2-DG transport and the lumped constant of Sokoloff's operational equation[53] are the same in normal and pathological conditions. However, several studies have shown that pathological conditions alter the value of the kinetic rate constants[31,45] and of the lumped constant.[29,45] Release of lysosomal enzymes in a pathological condition could hydrolyze phosphorylated glucose and 2-DG, which also would alter the lumped constant.[45] Because of the possible effects of stress and potential inaccuracy of the lumped constant in our model, comparison of the 2-DG results with cytochrome oxidase histochemical data, which avoids these problems, will be important.

We have established that a single administration of d-AMPH facilitates recovery on a specific locomotor task following a 400 g/cm^2 contusion injury, and therefore are attempting to elucidate the mechanism(s) by which this facilitation occurs. One must remember that the most likely action of AMPH is not directed toward the primary site of injury, but rather is affecting areas which are secondarily affected by the initial injury. Other possible explanations for recovery include:[26] 1) sprouting in the injured area or pruning in a remote area, 2) denervation supersensitivity, 3) sparing, and/or 4) disachisis, a remote functional depression.[19,25] It is unlikely that sprouting of nerve tissue or denervation supersensitivity account for the accelerated recovery, because both of these mechanisms require time for their occurrance, while the observed facilitation is seen on the first test 1 hour after d-AMPH administration.[21] The hypothesis of diaschisis proposes that functional changes occur in areas remote from the site of injury and these contribute to the symptoms. AMPH may alleviate some

injury-induced symptoms by affecting hypofunction in areas remote from the primary injury. There are data indicating that pharmacological stimulation of the CA system may "enable"[42] functionally depressed areas of an injured brain to respond to environmental stimulation, leading to an enhancement of recovery. Perhaps a single dose of the drug is effective because once "enabled", the functional depression is permanently removed. This remains to be firmly established. Spontaneous recovery of beam walking after sensorimotor cortex injury could be interpreted as a gradual dissipation of this hypofunction in such areas affected by the initial injury. Data bearing on this hypothesis are being collected.

As mentioned previously, AMPH has multiple actions within the central nervous system, one of which is on catecholaminergic (CA) neurotransmitters.[36] The fact that the AMPH facilitation of recovery is blocked by haloperidol, a CA antagonist,[21] indicates CA involvement in the action of AMPH. The important role for NE in recovery of function in our model is supported by the pharmacological data described in the last Annual Report. Antagonists of $alpha_1$-adrenergic receptors retarded recovery whereas antagonists of $alpha_2$-adrenergic receptors facilitated recovery. The $alpha_1$-antagonists, prazosin and phenoxybenzamine, reinstated the hemiplegic symptoms when administered 30 days post injury as did the $alpha_2$-agonist, clonidine.[61] (The rats had spontaneously recovered by day 16). The beta-adrenergic antagonist, propranolol, had no effect.

The LC is the major source of NE-containing neurons in the brain[27,42] with efferents projecting to the cortex and cerebellum, among other areas. Because of the evidence for involvement of the NE neurotransmitter system, it is possible that AMPH facilitates recovery by

stimulating the LC, causing release of NE from the LC terminal projections. Although AMPH inhibits LC firing, by activity at the alpha-2-autoreceptor,[27,30] it also induces release of NE and blocks reuptake.[36] The effect of stimulating massive release of NE appears to outweigh the inhibition of LC firing. L'Heureux, et al.[39] report a 450% d-AMPH induced increase of cortical NE above basal levels.

Recall that after sensorimotor cortex lesions only a single dose of d-AMPH is necessary to facilitate recovery of function on the beam-walking task in rats and cats.[21,22] A single dose of AMPH is similarly effective in hemiparetic stroke cases.[15] If AMPH facilitates recovery by inducing release of NE from LC terminals projecting to an area involved in the performance of the lost behavior, it is possible that this single "surge," combined with relevant experience, is sufficient to remove any functional depression and thereby facilitate recovery. Data from other studies indicate that NE is also protective against cell death[8] and facilitates recovery.[11] Robinson[51] reported decreased NE levels after cortical infarct in rats depending on the hemisphere injured. Interestingly, we also observed an unexpected and enduring decrease of cortical NE levels after cortical contusion. We have not compared left and right hemisphere injuries.

However, as discussed above, the results from these studies suggest that we must continue to consider alternative hypotheses. One essential consideration is the effect of AMPH on other neurotransmitters. We have no pharamacological data supporting involvement of DA in the facilitation of recovery in our model. DA is reported to alleviate symptoms in other brain injury models.[28,40,41] Apomorphine, tested in the sensorimotor cortex ablation model with a wide dose response range, was without effect

on recovery of function. However, while widely accepted as a postsynaptic DA agonist, apomorphine has been shown to act also as a presynaptic antagonist (by action at a DA autoreceptor).[2,14] AMPH also increases activity of serotonergic neurons, which have inhibitory projections to the LC.[43,44] The neurotransmitters acetylcholine and gamma-aminobutyric acid (GABA) are also affected by AMPH.[24] There is a powerful NE modulation of ACh release from the cortex (directly involving alpha-1 receptors) and this release is also mediated by GABA.[7,24] Because interactions between neurotransmitter systems are so complex,[52] it is very likely that AMPH is affecting these other transmitter systems in addition to NE, and that the inter-relationships between the systems are important to the overall affect of AMPH.

Our current data support the hypothesis that at least one aspect of the mechanism of AMPH facilitation of recovery is via the NE neurotransmitter system. While these data are suggestive, the basis of the AMPH effect on recovery of function remains unsolved.

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Table 1

Rating scale for assessing behavioral impairment on the beam-walking task.

7. Animal that can traverse the narrow elevated beam normally with no more than 2 footslips of the left hindlimb.

6. Animal that is able to locomote across the beam using the left hindlimb to aid in more than 50% of its steps on the beam.

5. Animal that can traverse the beam while using the left hindlimb to aid less than 50% of its steps on the beam.

4. Animal that can traverse the beam, placing the foot of the left hindlimb on the horizontal surface of the beam without using the limb to aid in forward locomotion.

3. Animal that can traverse the beam while dragging the affected hindlimb or showing treading/stepping motions with the left hindlimb, but is not capable of placing the left hindfoot on the horizontal surface of the beam during the traversal.

2. Animal that is unable to traverse the beam, but is able to pace the left hindlimb on the beam.

1. Animal that is unable to traverse the beam or place the left hindlimb on the beam.

Table 2

LOCUS COERULEUS

NOREPINEPHRINE (ng/g wet wt)

HEMISPHERE			Treatment Group			
			Sham/Saline (n=5)	Sham/AMPH (n=4)	Injury/Saline (n=8)	Injury/AMPH (n=9)
	Right	X	856.0	920.0	817.5	827.5
		S.E.M.	52.3	109.5	49.5	46.4
	Left	X	636.0	860.0	817.5	857.5
		S.E.M.	110.0	92.5	72.3	59.7

Table 2: Absolute levels of NE in the LC expressed in ng/g wet weight of tissue for right and left hemispheres for each treatment group. Data are expressed as mean ± S.E.M.

Table 3

VENTRAL TEGMENTUM

NOREPINEPHRINE (ng/g wet wt)

HEMISPHERE			Treatment Group			
			Sham/Saline (n=5)	Sham/AMPH (n=4)	Injury/Saline (n=8)	Injury/AMPH (n=9)
	Right	X	465.0	440.0	485.7	462.0
		S.E.M.	45.9	45.8	35.5	32.3
	Left	X	515.0	420.0	375.0	460.0
		S.E.M.	60.8	45.1	47.6	47.0

Table 3: Absolute levels of NE in the ventral tegmentum expressed in ng/g wet weight of tissue for right and left hemispheres for each treatment group. Data are expressed as mean ± S.E.M.

Table 4

CEREBELLAR CORTEX

NOREPINEPHRINE (ng/g wet wt)

HEMISPHERE			Treatment Group			
			Sham/Saline (n=5)	Sham/AMPH (n=4)	Injury/Saline (n=8)	Injury/AMPH (n=9)
	Right	X	179.8	115.0	64.0	96.9
		S.E.M.	105.1	26.3	10.1	2.3
	Ratio NE/MHPG		47.3	4.2	1.6	3.5
	Left	X	96.4	95.5	87.7	104.9
		S.E.M.	17.0	19.0	12.9	8.3
	Ratio NE/MHPG		2.3	2.5	2.2	2.1

MHPG (ng/g wet wt)

HEMISPHERE						
	Right	X	3.8	27.1	40.7	27.6
		S.E.M.	0.8	9.8	8.9	6.6
	Left	X	41.8	37.6	39.9	49.9
		S.E.M.	16.0	15.2	13.8	18.4

Table 4: Absolute levels of NE and MHPG in right and left cerebellar cortex. The ratio of the means between NE and MHPG are also given. Data are displayed as mean ± S.E.M.

Table 5

VENTRAL TEGMENTUM

DOPAMINE (ng/g wet wt)

Treatment Group

HEMISPHERE			Sham/Saline (n=5)	Sham/AMPH (n=4)	Injury/Saline (n=8)	Injury/AMPH (n=9)
	Right	X	450.0	420.0	357.0	377.5
		S.E.M.	44.3	87.2	53.9	53.4
	Ratio DA/Dopac		7.5	4.7	7.3	8.1
	Left	X	433.0	405.0	295.0	422.2
		S.E.M.	40.2	82.3	51.5	75.0
	Ratio DA/Dopac		7.8	6.2	6.7	5.2

VENTRAL TEGMENTUM

DOPAC

Treatment Group

HEMISPHERE			Sham/Saline (n=5)	Sham/AMPH (n=4)	Injury/Saline (n=8)	Injury/AMPH (n=9)
	Right	X	60.0	90.0	48.6	46.7
		S.E.M.	14.1	40.4	12.0	19.2
	Left	X	55.6	65.0	43.8	81.1
		S.E.M.	18.7	32.8	12.7	24.0

Table 5: Absolute levels of DA and DOPAC in right and left ventral tegmentum. The ratio of the means between DA and DOPAC are also given. Data are displayed as mean ± S.E.M.

Table 6

GLUCOSE UTILIZATION RATES (umol/100g/min) 16 DAY TIME POINT

Nucleus Acumbens		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	34.3	20.2	89.3	68.4
		111.4	130.6	49.3	63.7
	R	37.1	18.3	90.7	69.7
		109.7	129.7	50.7	62.2
Caudate Putamen		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	30.3	17.2	60.4	45.5
		96.7	108.6	45.3	58.1
	R	37.7	18.2	63.1	50.4
		100.2	117.9	45.4	57.5
Globus Pallidus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	42.4	19.7	65.6	68.1
		96.3	121.1	43.4	60.0
	R	31.6	19.9	70.3	74.4
		99.5	120.3	46.4	58.7
Subthalamic Nucleus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	30.9	19.1	74.4	57.2
		114.1	176.4	52.8	78.9
	R	32.3	21.8	70.7	64.8
		114.1	181.7	52.9	80.9
Substantia Nigra		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	40.7	14.0	71.2	65.2
		114.8	134.3	54.6	75.2
	R	42.1	18.0	69.1	60.8
		114.3	145.4	53.2	73.0
Lateral Geniculate Nucleus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	35.8	17.8	66.6	63.1
		93.8	132.1	49.9	71.5
	R	31.7	19.1	65.4	61.0
		88.7	128.6	49.1	68.9

Red Nucleus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	37.4	17.3	72.9	44.1
		111.1	123.5	45.2	66.2
	R	41.3	18.5	71.6	44.8
		101.8	122.4	40.4	67.2
Medial Geniculate Nucleus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	42.4	11.9	68.8	35.6
		100.7	106.6	47.3	64.4
	R	32.3	14.3	66.7	31.1
		104.6	121.6	45.3	58.5
Posterior Neocortex (caudal to injury)		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	45.9	16.5	65.4	40.4
		103.3	105.2	45.3	66.3
	R	57.5	25.3	64.6	50.5
		113.7	160.7	43.3	67.2
Superior Colliculus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	45.1	17.4	81.1	65.3
		106.7	144.1	49.4	70.1
	R	50.8	20.7	84.7	59.2
		109.4	145.9	47.3	71.5
Inferior Colliculus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	36.7	19.3	78.1	36.7
		109.3	118.7	52.4	63.6
	R	37.6	18.9	80.8	27.9
		116.2	97.2	54.5	58.8
Locus Coeruleus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	42.1	24.1	99.6	65.8
		94.1	168.6	56.7	63.3
	R	41.9	22.3	75.4	65.8
		101.4	171.4	56.1	66.8

Dorsal Tegmental Nucleus		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	35.5	15.7	85.5	54.8
		92.0	131.5	47.4	66.2
	R	36.6	14.8	85.9	46.1
		104.5	121.4	45.0	62.1
Superior Cerebellar Peduncle		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	50.8	170.5	93.0	87.7
	R	49.8	164.0	97.4	78.8
Cerebellum (Gray Matter)		Contusion \AMPH	Contusion Sal	Sham AMPH	Sham Sal
	L	53.1	24.9	99.0	85.6
		119.9	141.5	62.9	80.4
	R	52.9	25.1	96.7	79.7
		114.9	143.1	63.1	80.5

Table 6: Glucose utilization rates for 16 day time point (average of 2 animals). Note that with our particular software, low values represent high glucose utilization rates whereas high volumes represent low glucose utilization rates.

List of Figures

Figure 13: Mean beam-walk scores versus time for animals that received a 400 gm/cm contusion and intraventricular injection of saline or NE.

Figure 14: Mean beam-walk scores versus time for animals that received a 400 gm/cm contusion and intraventricular injection of saline or DA.

LOCUS COERULEUS EXPERIMENTAL PROTOCOL

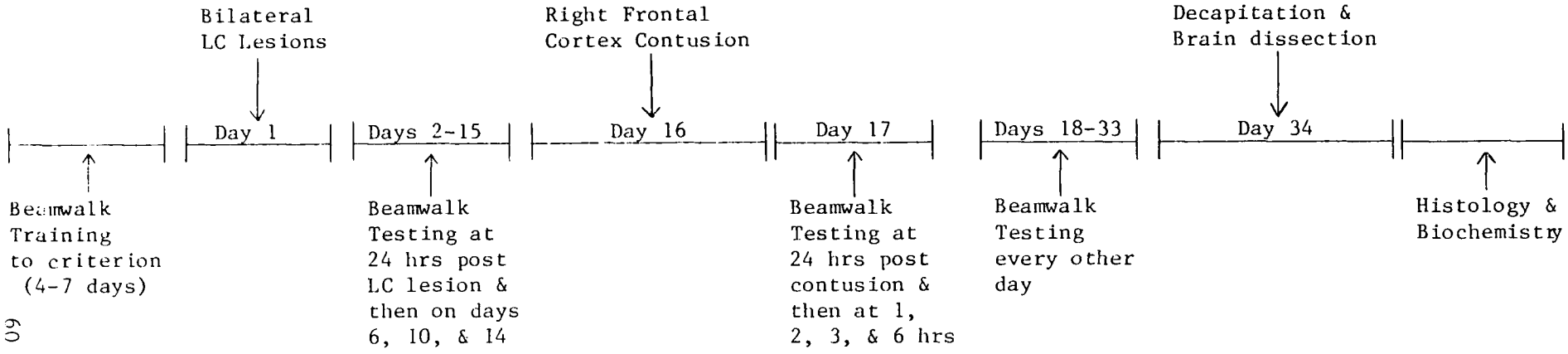


Figure 1

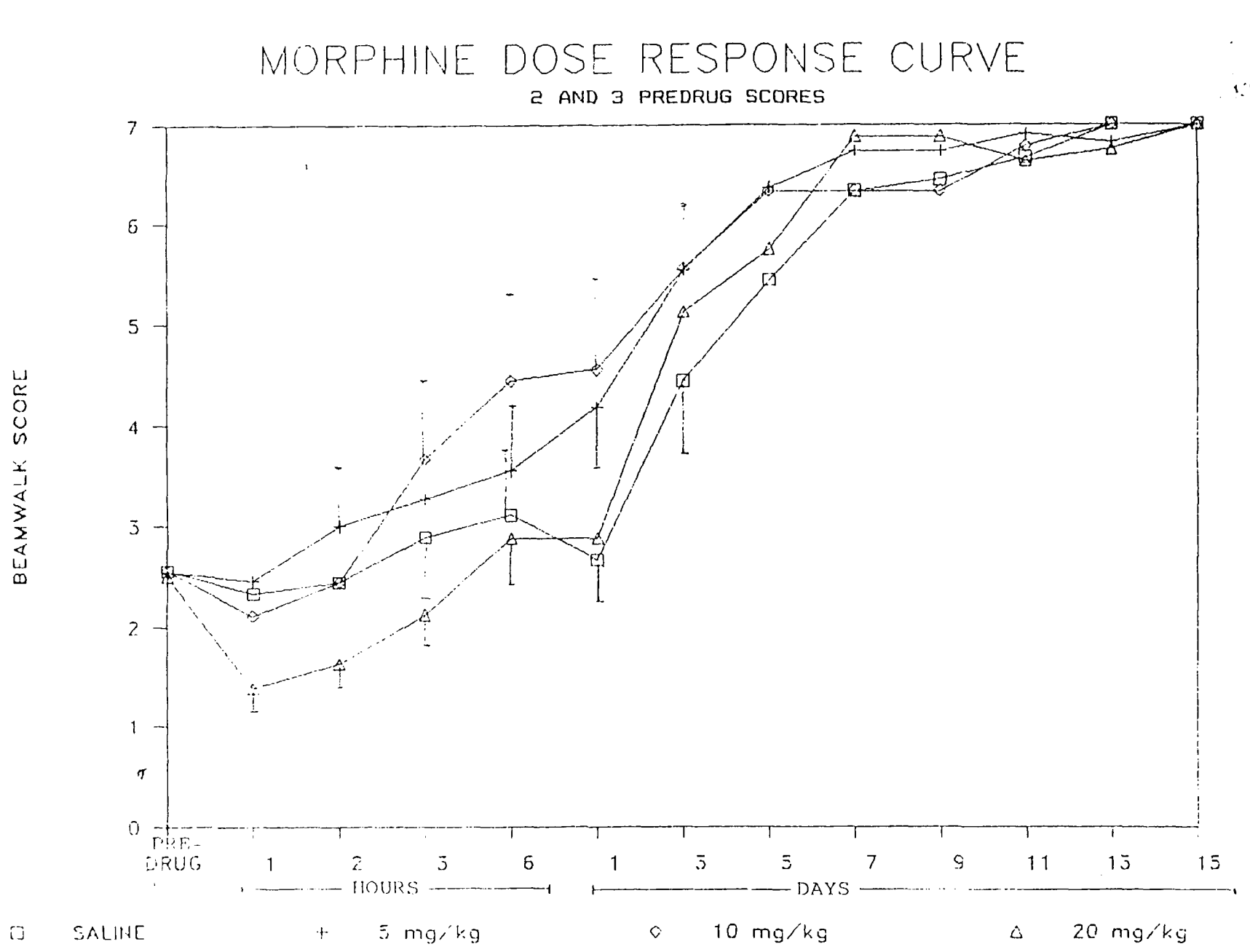

MORPHINE DOSE RESPONSE CURVE
2 AND 3 PREDRUG SCORES
BEAMWALK SCORE
0
1
2
3
4
5
6
7
PRE-DRUG
1
2
3
6
1
3
5
7
9
11
13
15
HOURS
DAYS
SALINE
5 mg/kg
10 mg/kg
20 mg/kg

Figure 2

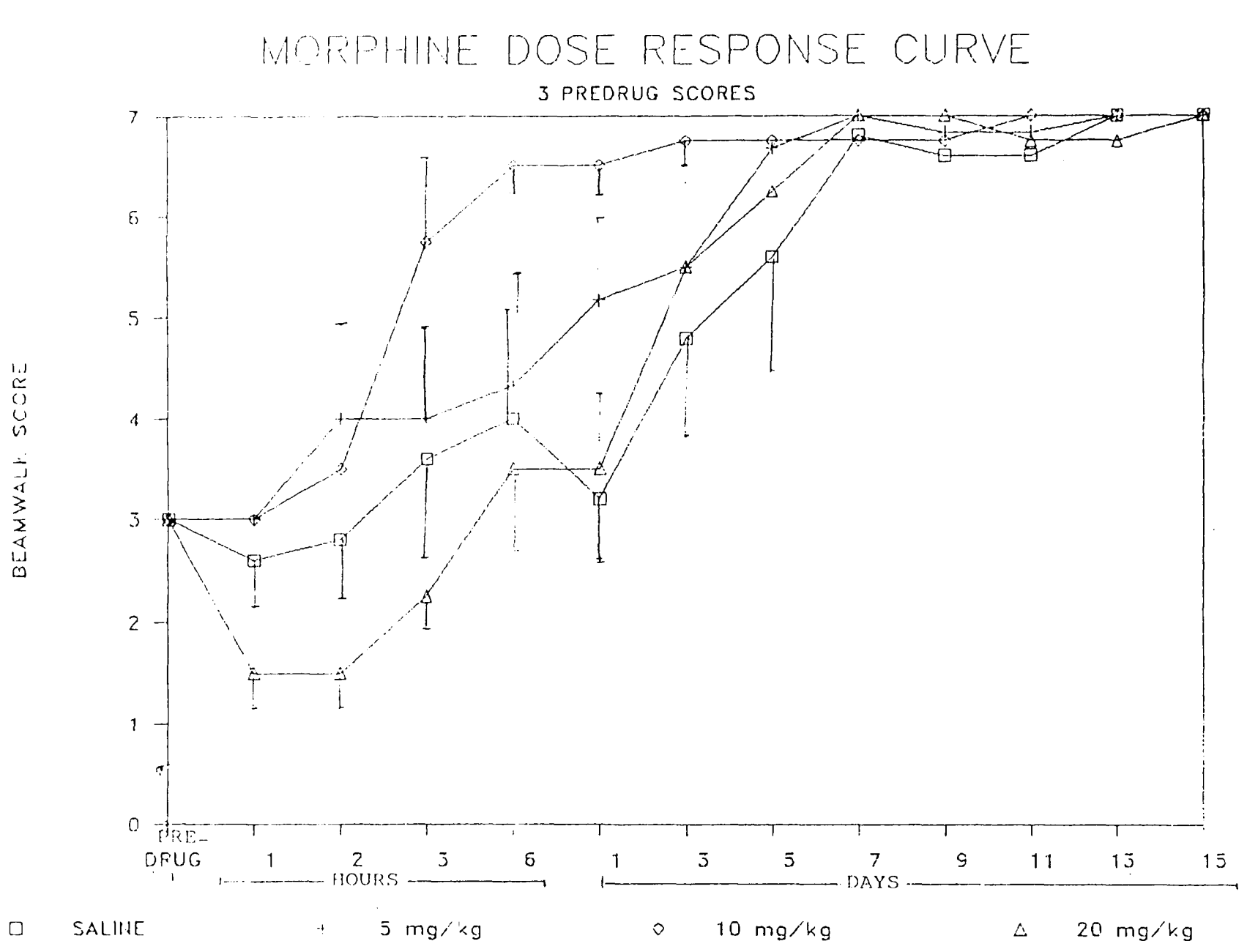

MORPHINE DOSE RESPONSE CURVE
3 PREDRUG SCORES
BEAMWALK SCORE
0
1
2
3
4
5
6
7
PRE-DRUG
1
2
3
6
1
3
5
7
9
11
13
15
HOURS
DAYS
SALINE
5 mg/kg
10 mg/kg
20 mg/kg

Figure 3

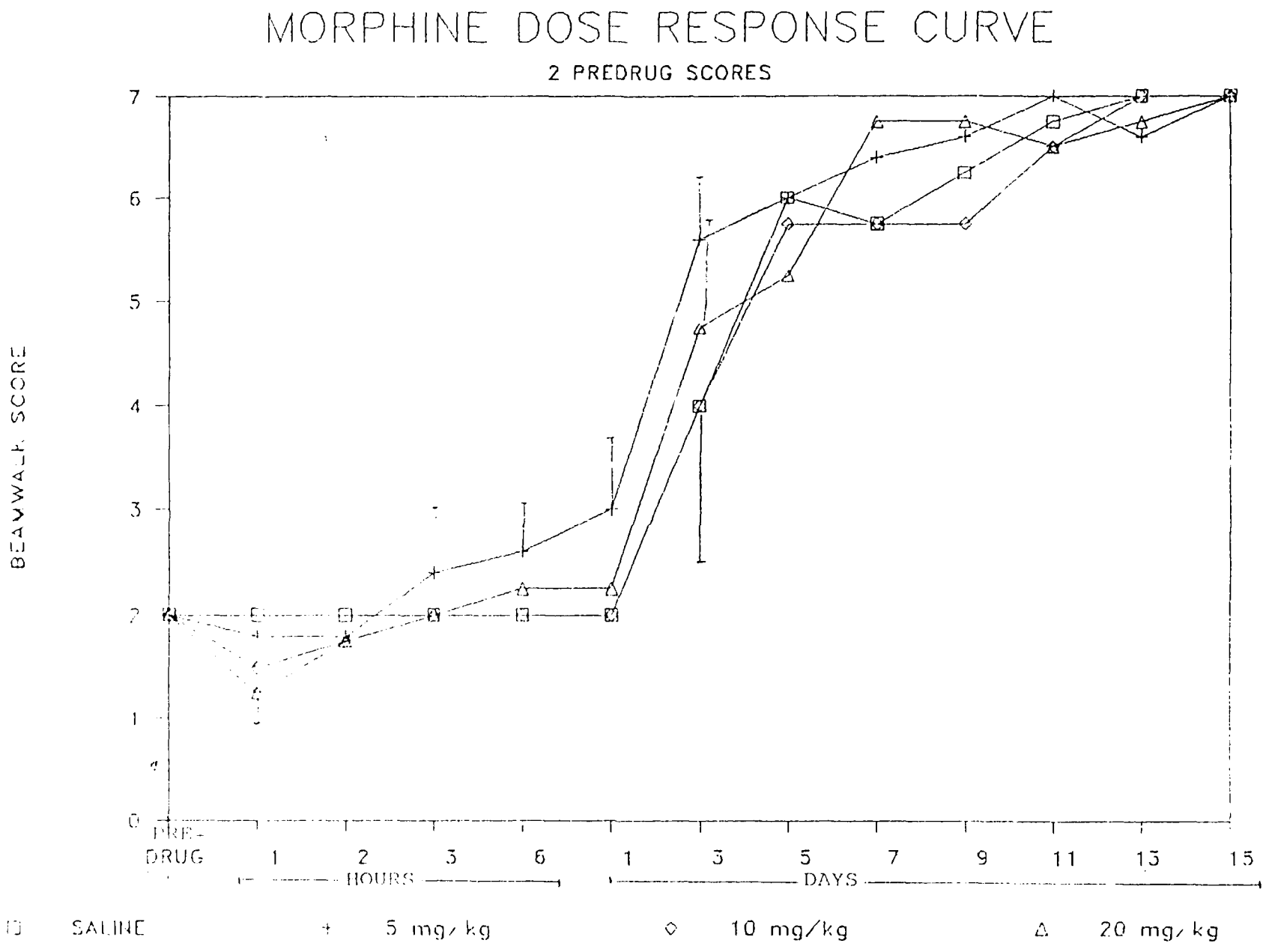
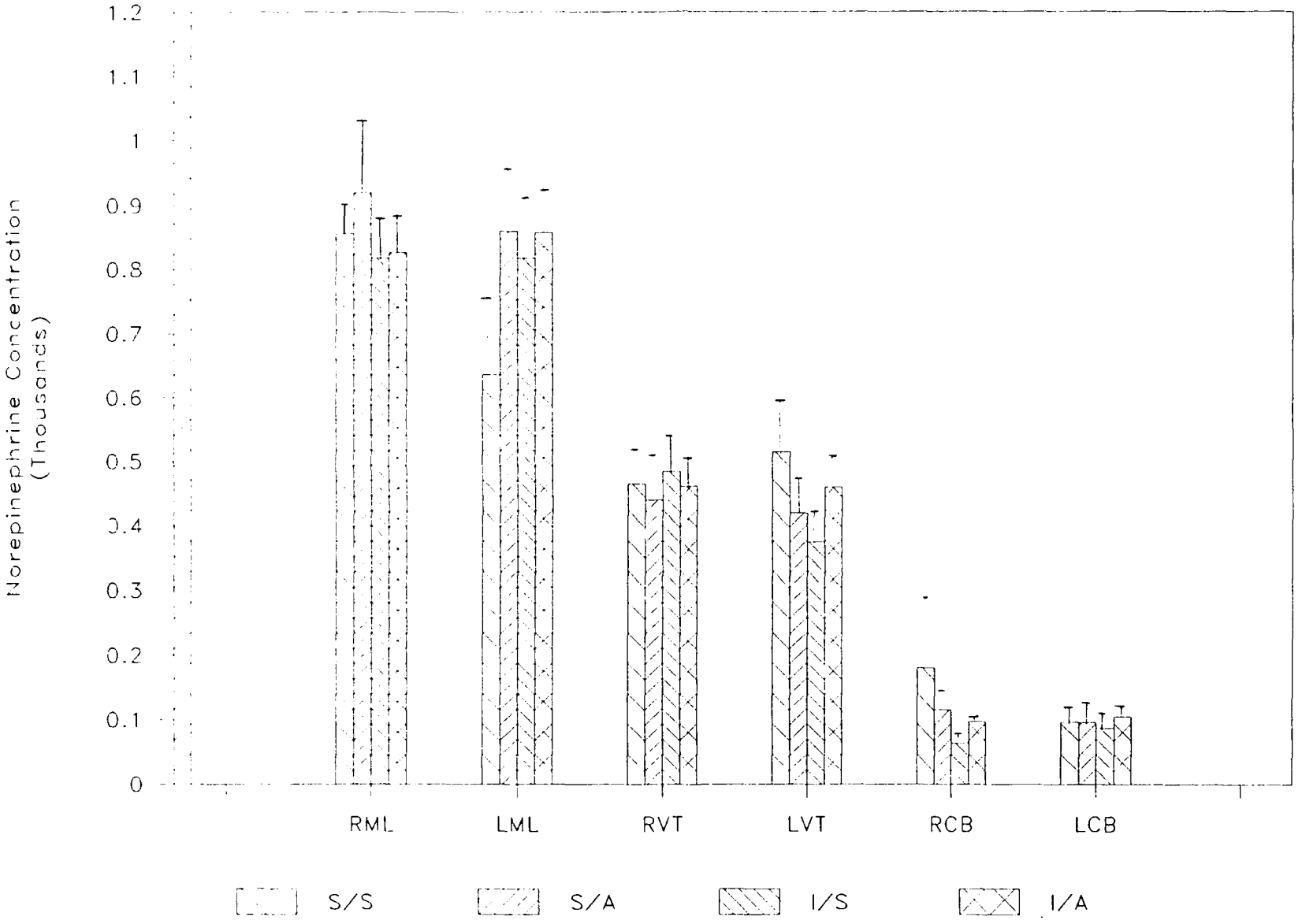

MORPHINE DOSE RESPONSE CURVE
2 PREDRUG SCORES
BEAM WALK SCORE
0
1
2
3
4
5
6
7
PRE-DRUG
1
2
3
6
1
3
5
7
9
11
13
15
HOURS
DAYS
SALINE
5 mg/kg
10 mg/kg
20 mg/kg

Figure 4


Norepinephrine Concentration
(Thousands)
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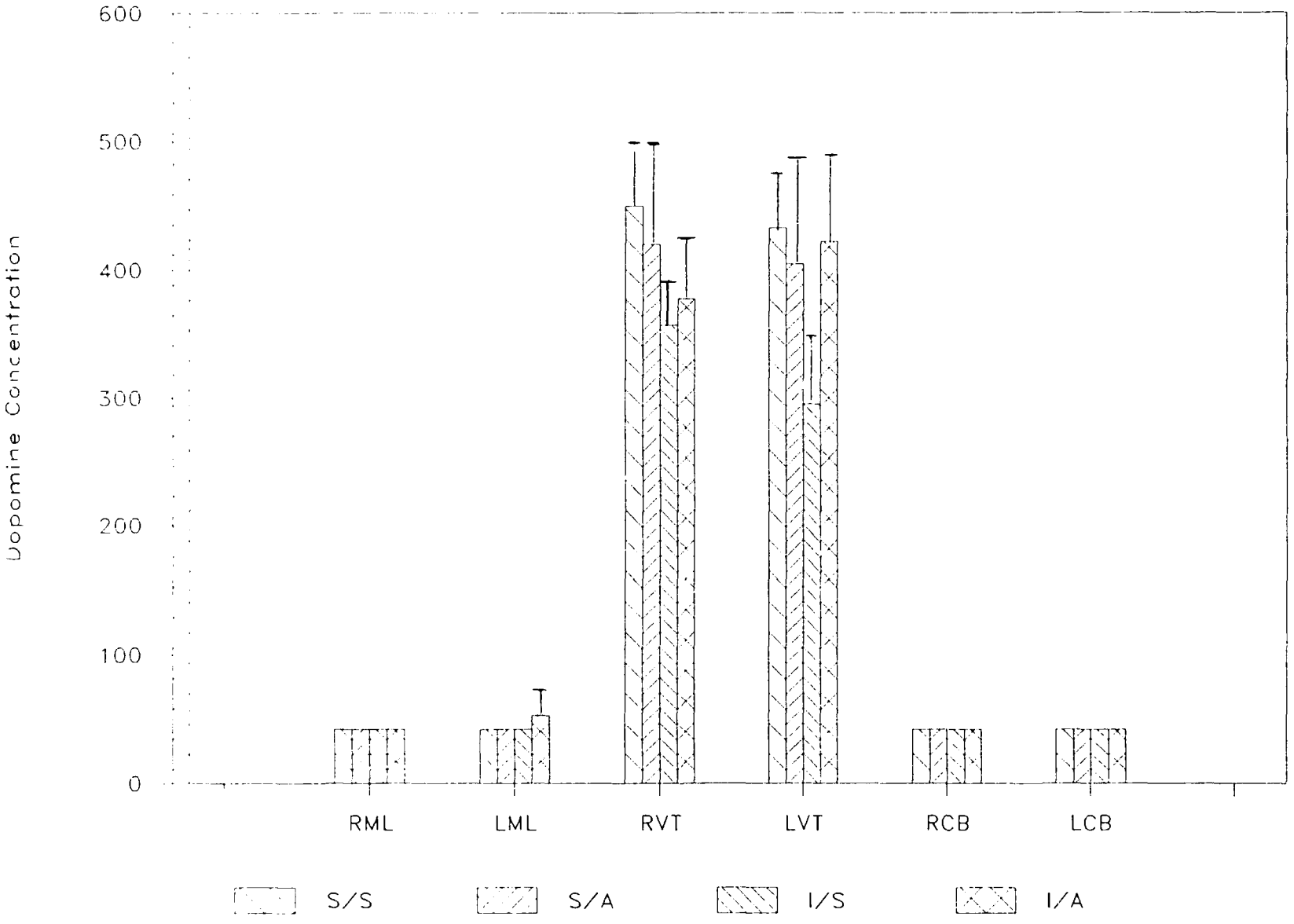
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
RML
LML
RVT
LVT
RCB
LCB
S/S
S/A
I/S
I/A

Figure 5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400
300
200
100
0
Dopomine Concentration
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RML
LML
RVT
LVT
RCB
LCB
S/S
S/A
I/S
I/A

Figure 6

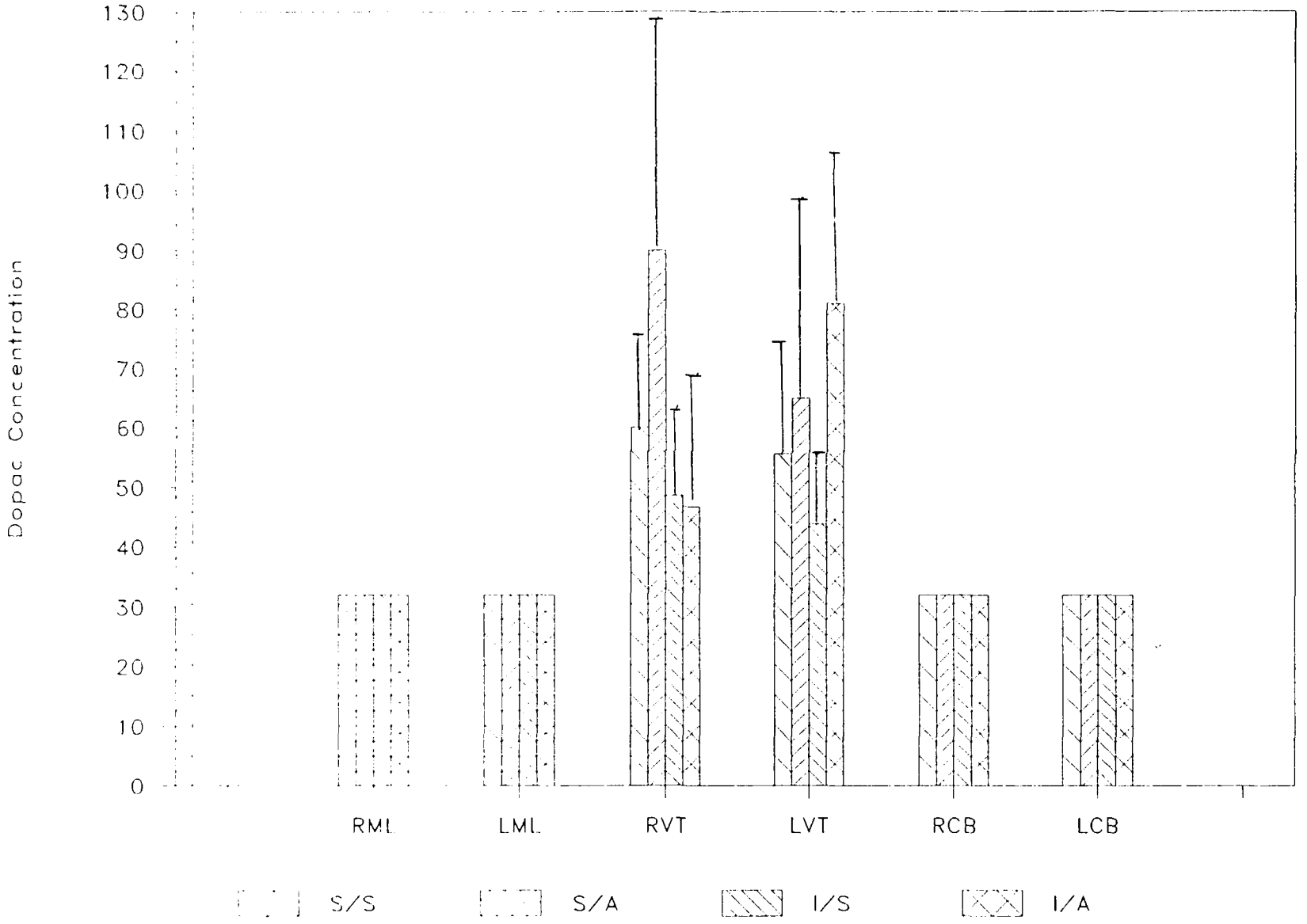
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Dopac Concentration
99
RML
LML
RVT
LVT
RCB
LCB
S/S
S/A
I/S
I/A

Figure 7

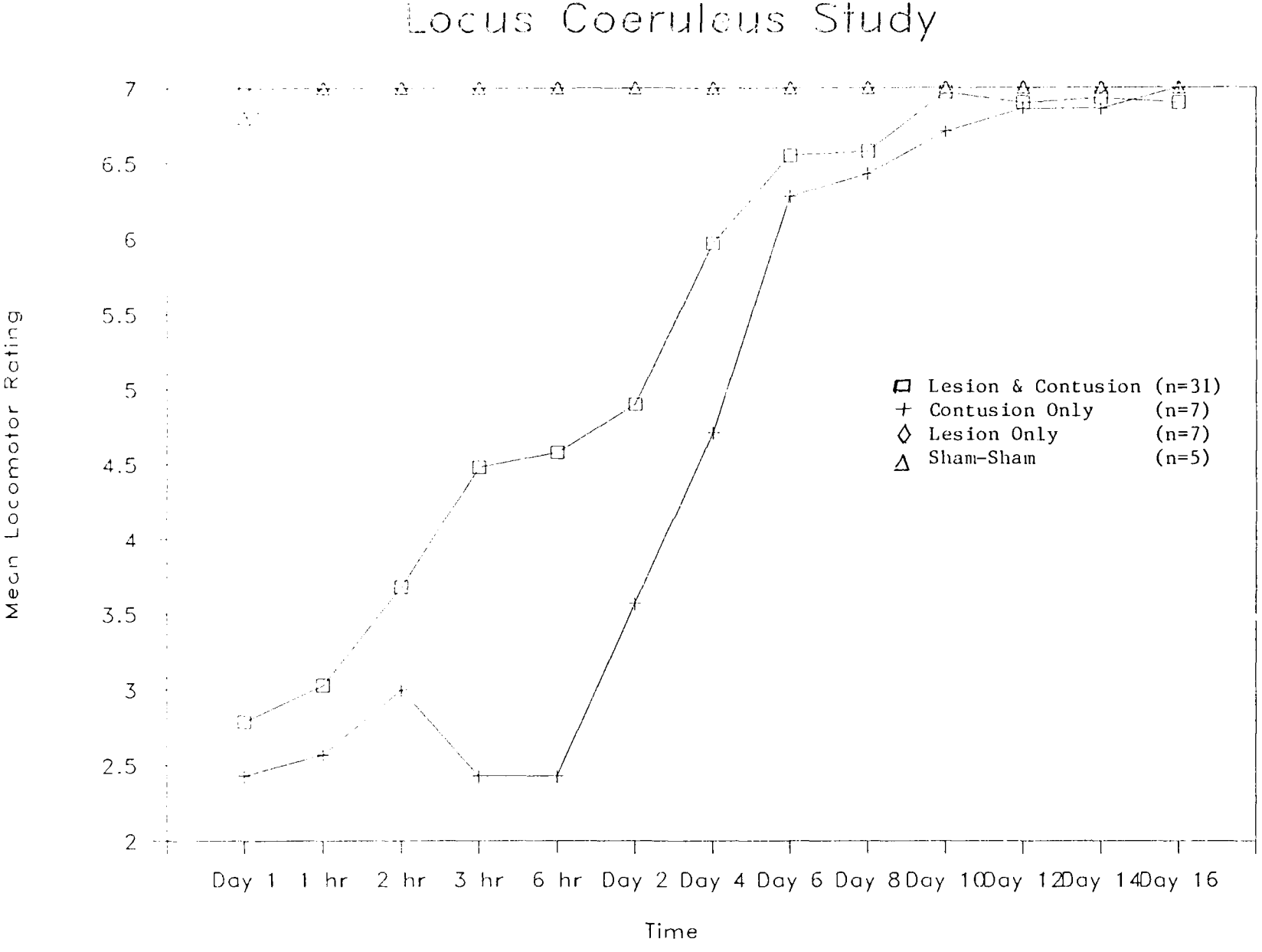
Locus Coeruleus Study
Mean Locomotor Rating
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
Lesion & Contusion (n=31)
Contusion Only (n=7)
Lesion Only (n=7)
Sham-Sham (n=5)
Day 1
1 hr
2 hr
3 hr
6 hr
Day 2
Day 4
Day 6
Day 8
Day 10
Day 12
Day 14
Day 16
Time

Figure 8

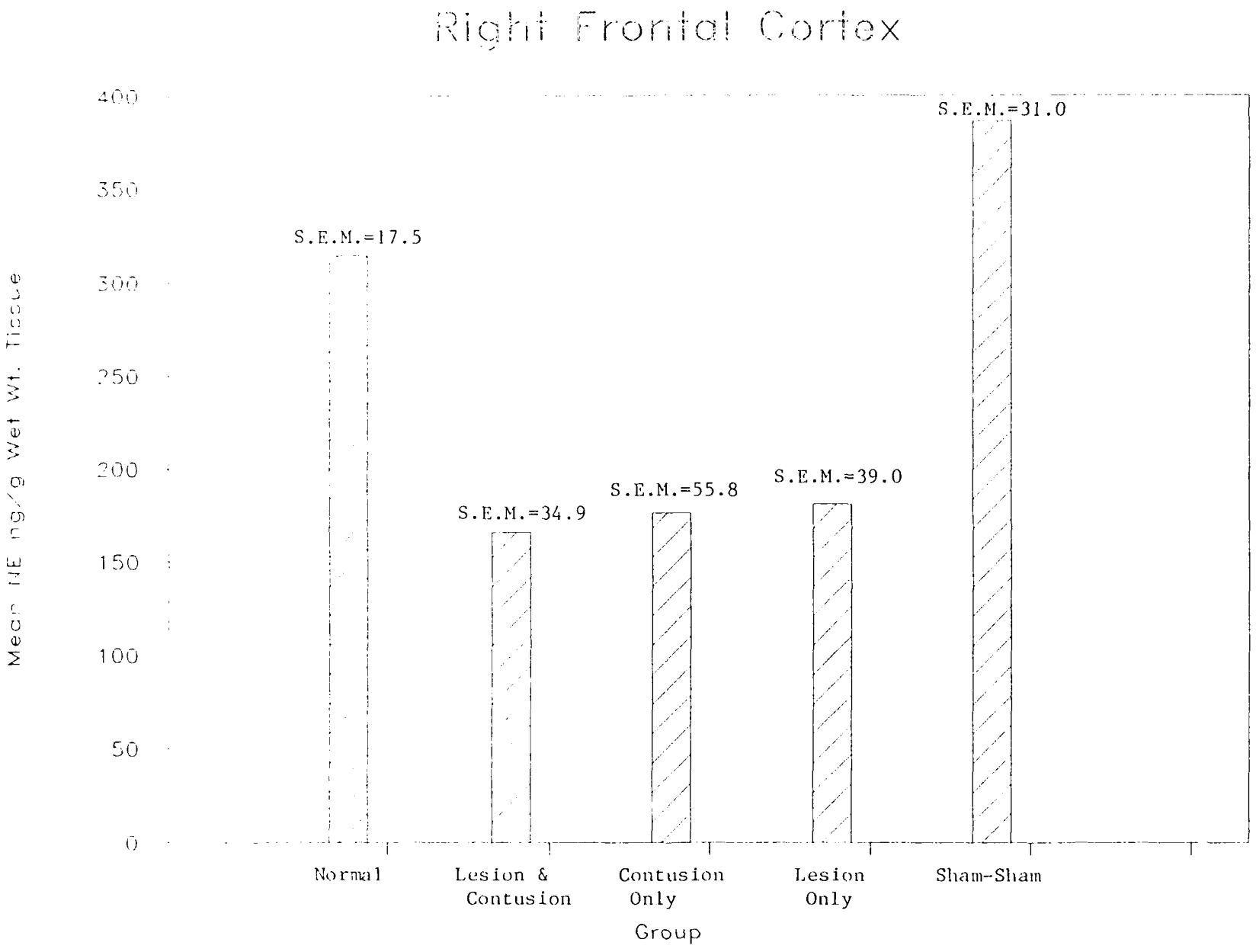
Right Frontal Cortex
Mean NE ng/g Wet Wt. Tissue
400
350
300
250
200
150
100
50
0
S.E.M.=17.5
S.E.M.=34.9
S.E.M.=55.8
S.E.M.=39.0
S.E.M.=31.0
Normal
Lesion & Contusion
Contusion Only
Lesion Only
Sham-Sham
Group

Figure 9

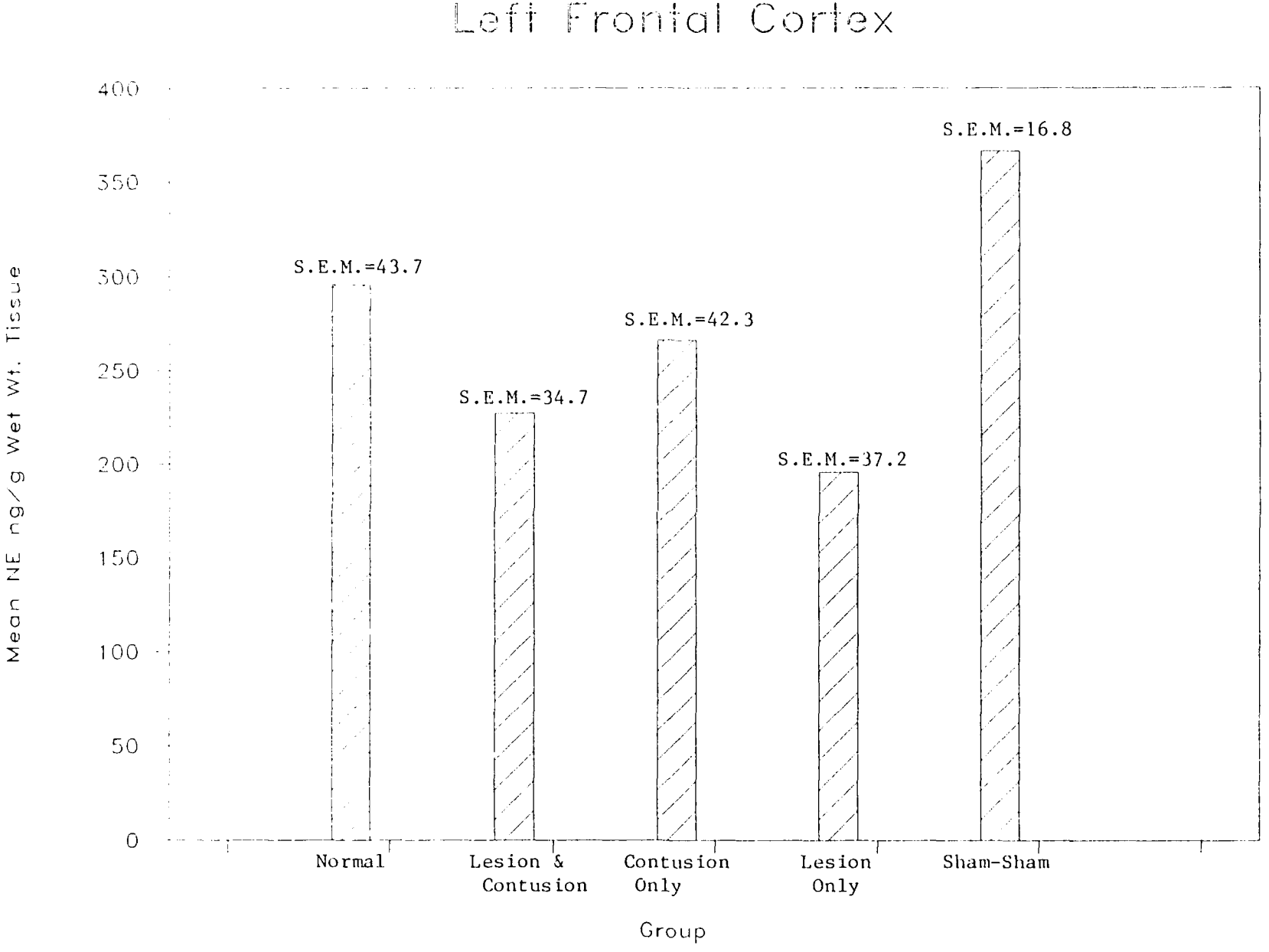
Left Frontal Cortex
Mean NE ng/g Wet Wt. Tissue
400
350
300
250
200
150
100
50
0
S.E.M.=43.7
S.E.M.=34.7
S.E.M.=42.3
S.E.M.=37.2
S.E.M.=16.8
Normal
Lesion & Contusion
Contusion Only
Lesion Only
Sham-Sham
Group

Figure 10

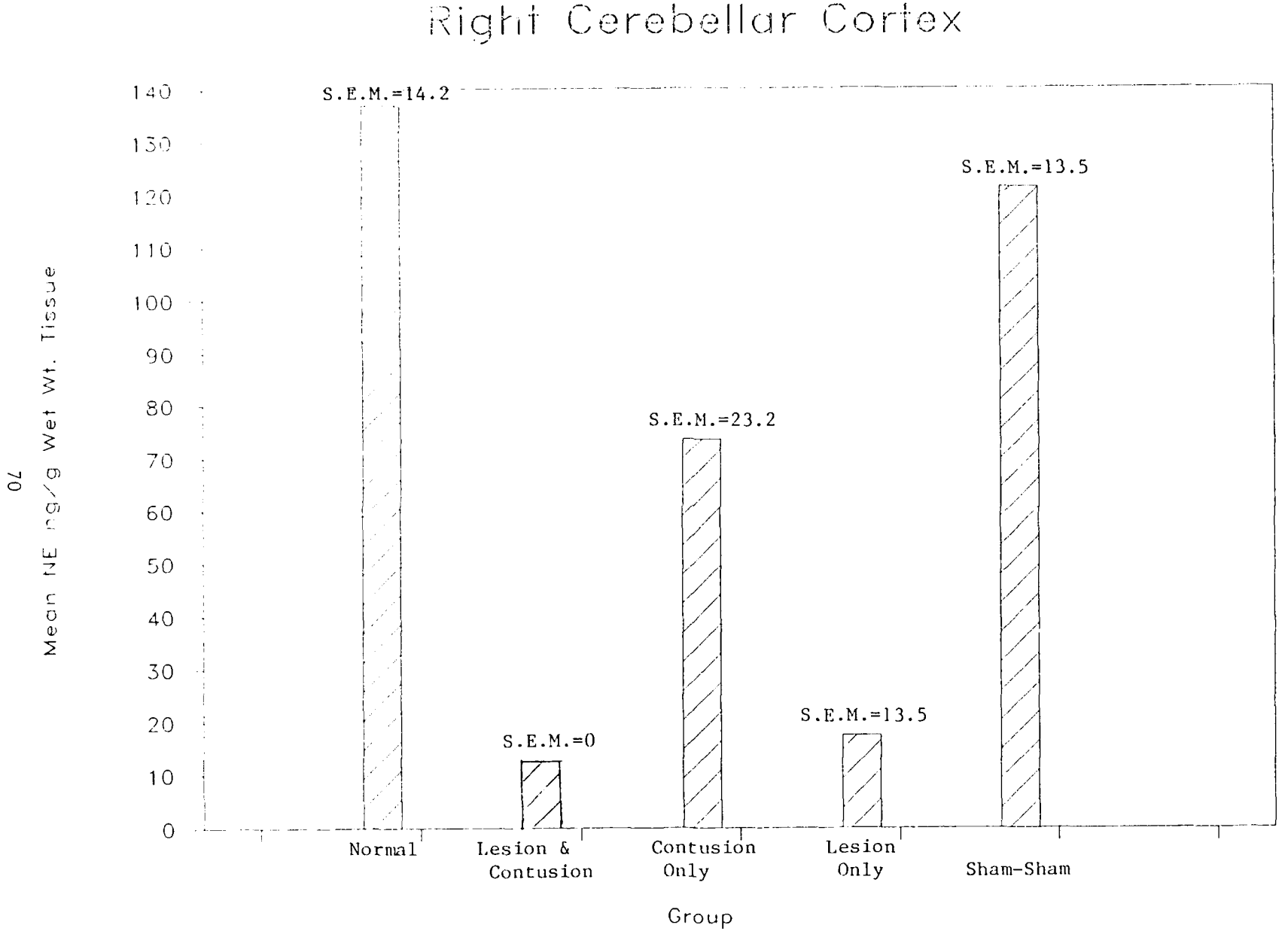
Right Cerebellar Cortex
Mean NE ng/g Wet Wt. Tissue
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
S.E.M.=14.2
S.E.M.=0
S.E.M.=23.2
S.E.M.=13.5
S.E.M.=13.5
Normal
Lesion & Contusion
Contusion Only
Lesion Only
Sham-Sham
Group

Figure 11

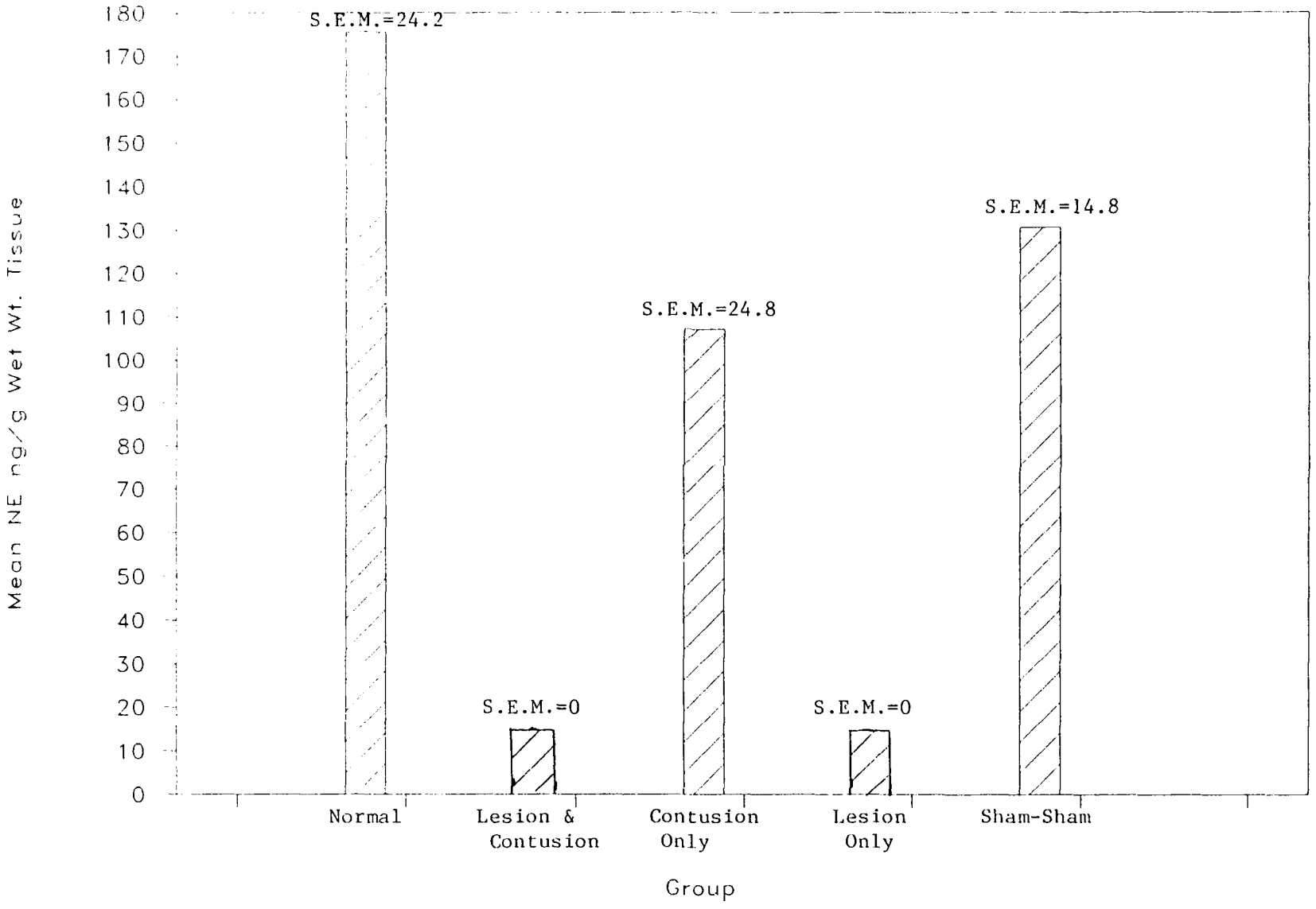
Left Cerebellar Cortex
Mean NE ng/g Wet Wt. Tissue
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
S.E.M.=24.2
S.E.M.=0
S.E.M.=24.8
S.E.M.=0
S.E.M.=14.8
Normal
Lesion & Contusion
Contusion Only
Lesion Only
Sham-Sham
Group

Figure 12

Norepinephrine

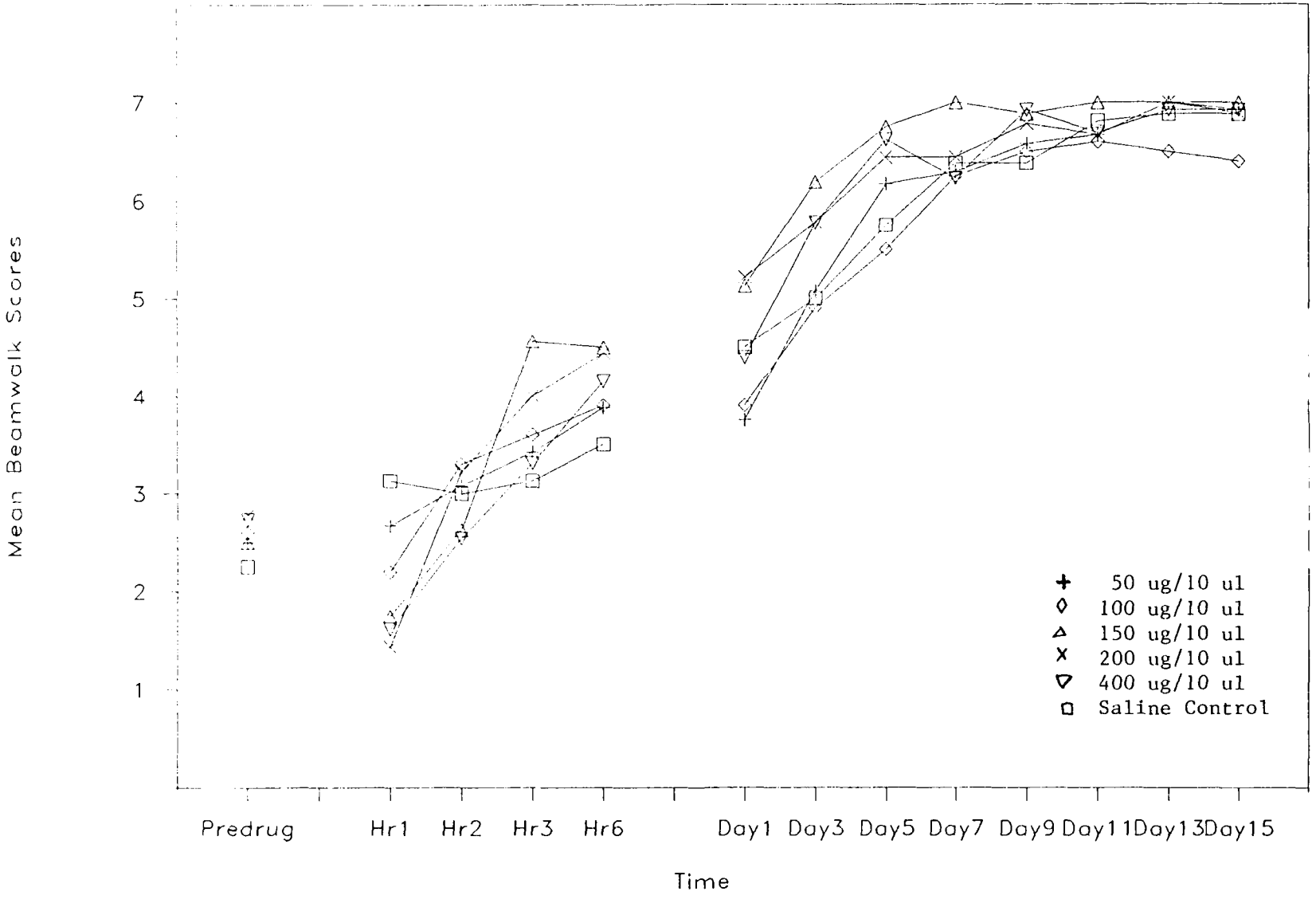

Figure 13

Dopamine

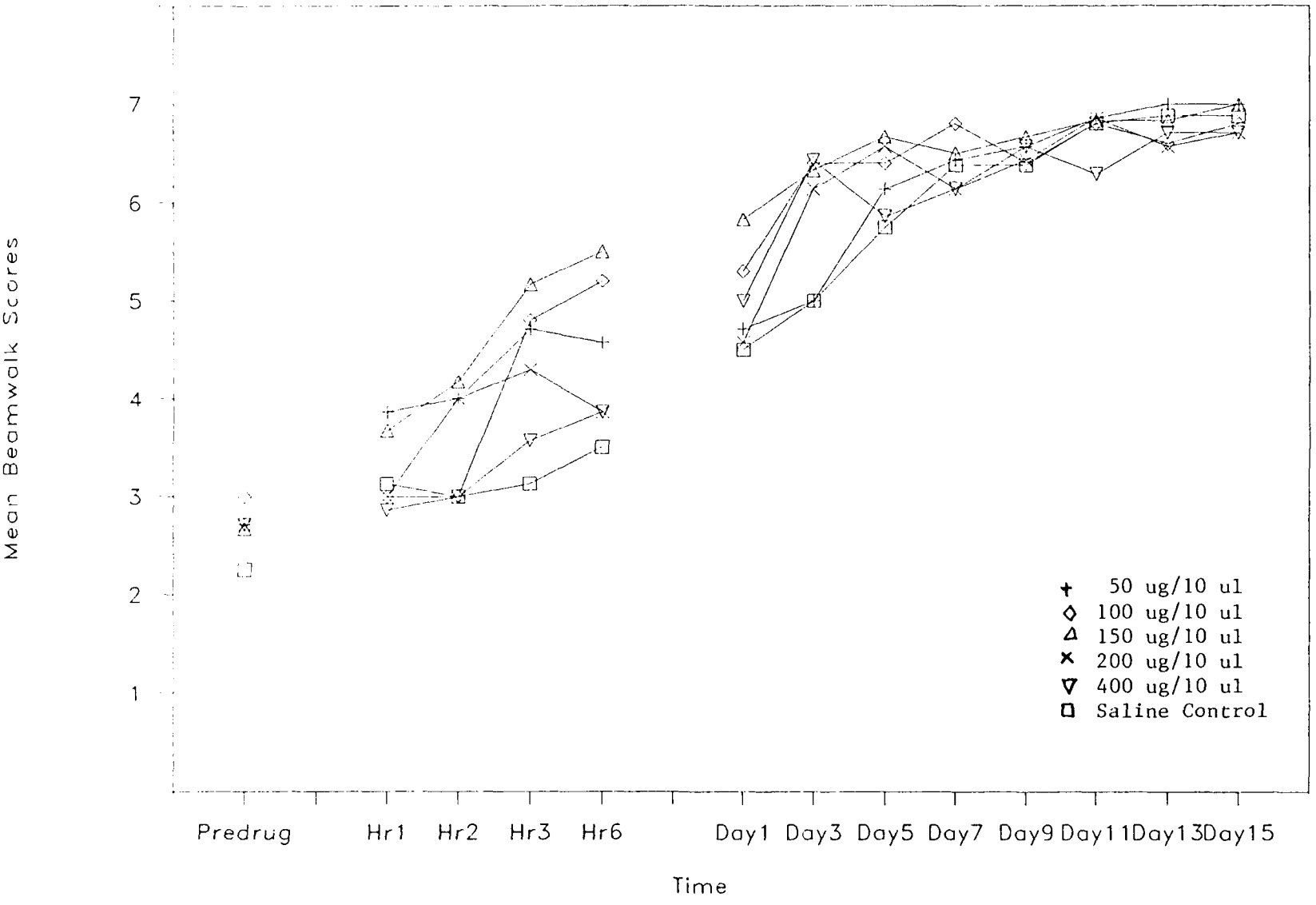

Figure 14

END

DATE

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